QUATERNARY AMMONIUM COMPOUNDS

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UNITED TRADE PRESS LTD.

	Date of	Date of

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IN CHEMICAL STERILISATION

by

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UNITED TRADE PRESS, LTD.
24 BRIDE LANE, LONDON, E.C.4
1951

To

Mr. L. P. James and My Colleagues
in The British Hydrological Corporation

ACKNOWLEDGMENTS

I wish to thank the Editors of several journals for permission to reproduce Tables, and Dr. J. G. Davis, D.Sc., Mr. H. Bunker, M.A., and Mr. G. R. A. Short, Ph.C., for valuable advice and criticism.

PREFACE

In writing this small book I have tried to present the essential facts about the properties and uses of the quaternary ammonium germicides to the wide range of people interested in them. This includes not only chemists and bacteriologists working in the food industries, to whom certain chapters are more specifically directed, but also executives and process department managers in food and beverage plants.

The personnel responsible for the efficient working of plant and for production may not be specialised as chemists or bacteriologists, but they are frequently men whose appreciation of a wide range of technical and semi-technical problems is often quite remarkable, and they will want to know not only the possibilities, but the limitations, of this new type of sterilising compound.

Public health authorities and sanitary inspectors are also interested in the use of these substances, and here again I have felt that there is need for an explanatory approach.

I am well aware of the dangers which beset an attempt to give a book such as this a wide appeal, and that a certain amount of criticism is likely to be levelled on this account.

Chapters IV and V are particularly written for chemists and bacteriologists interested in food plant hygiene, although these also include matter of general interest, and I hope that the book will be accepted as an approach to the practical aspects of the use and development of an important class of germicide.

J. C. L. RESUGGAN.

Worcester Park,
Surrey.
March 1951.

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CHAPTER I

INTRODUCTION

CHEMICAL sterilising of food plant and equipment has, for a long time, been an important way of achieving, if not complete sterility, at any rate a considerable lowering of the bacterial population. Sometimes chemical methods of sterilisation have replaced heat altogether, but in other cases they have been supplementary to the use of steam or boiling water. In certain cases where heat-resistant organisms are found, chemical sterilising can prove a most effective means of removing this cause of trouble; but on the other hand, it is generally admitted that spores of many kinds resist chemical bactericides to a greater degree than they do heat.

The intelligent use of all sterilising methods lies in applying those which are best suited to the conditions which obtain in any given set of circumstances, and it is not at all uncommon to find heat, and sometimes more than one chemical bactericide, being used in one food factory or plant. Changes in microbiological flora also take place from time to time, and this may necessitate some fresh means of attack, while wear and corrosion of plant may also present problems which require some new approach. It is, therefore, reasonable to admit all possible methods of sterilising, and to apply them where their use achieves the best result with the greatest economy.

Heat in various forms is still the most extensive sterilising medium in use, and confining these remarks to food and beverage processing and production plants, it would be true to say that chlorine in various forms, such as sodium hypochlorite solutions and chloramines, probably occupies the next place on the list, while other substances such as hydrogen peroxide, sodium bi-sulphite and even phenols are often used, although the conditions in the latter case may be unusual.

One of the first considerations in the choice of a chemical bactericide for this kind of work is, of course, the question of toxicity and the possibility of causing food or beverages to have taints or a flavour. Next must come the effect of the chemical sterilising agent upon the plant, and this involves consideration of the nature of the surface concerned, in case the metal is susceptible to corrosion by the particular chemical agent whose use is contemplated.

The latest addition to the ranks of chemical sterilising agents is the quaternary ammonium compounds which have, in the past few years, achieved considerable prominence.

Scientists in the United States of America first took up the development of this new type of bactericide, and from 1943 onwards a considerable literature began to accumulate concerning their properties and uses. The main advantages claimed were low

toxicity, odourlessness, high penetrating power and good killing powers against various micro-organisms at concentrations often as low as 1 part in 10,000 of water.

The advent of the new sterilising agent was not by any means received with universal approbation; indeed, such a state of affairs could not have been reasonably expected, firstly because the older type of sterilising compound regarded its position as being jeopardised, and secondly because the scientists were by no means satisfied that the properties claimed for the new compounds had been sufficiently proved. In the technical literature a controversy broke out, which in one or two extreme cases even became rather acrimonious, as the facts for and against the new substances were debated. This controversy has proved to be of the greatest value, and has guided workers in this field into fresh lines of approach, which have yielded valuable data as to the mode of action and the limitations of the quaternary ammonium compounds. Side by side with the scientific discussions, development proceeded, and as a result about twenty preparations are freely available in the United States of America.

Although the medical field has not been neglected with this type of compound, it is in the direction of food and beverages that these substances have received by far the greatest attention.

Sterilisation of various kinds of food and beverage plant, kitchen utensils, drinking-glasses, ice cream equipment, has provided important problems which the quaternary ammonium compounds seemed admirably suited to solve. It seems without question that the development and use of these bactericides will be extended, and in this country the interest in them has, during the past three years, become quite considerable. It is important for workers in the food industry to appreciate both the advantages and the limitations of the quaternary ammonium compounds, in order that they may be applied most usefully, and with the assurance that the best possible results are being obtained bacteriologically for the particular circumstances involved.

At the time of writing (1951), the earlier problems connected with the anti-bacterial activity of these substances appear largely to have been solved. Attention is now directed to the question of toxicity, the main consideration being whether or not minute traces of these compounds can have any harmful cumulative effect in the human body. That is a question which can be asked about thousands of substances with which our skin and mucous surfaces come in contact regularly, and it is necessary to keep an open mind while awaiting the necessary accumulation of evidence which may take years. Nevertheless there does appear to have been a tendency in some quarters to take the matter out of its proper perspective by laying undue emphasis on certain more or less academic points.

Naturally, the fact that these germicides are relatively new, and in many cases untried, is bound to focus critical attention upon

them to a degree which would not occur with substances which have been in use for many years whether sterilising compounds, additives to food, colouring matters or essences.

An interesting paper by J. B. M. Coppock[42] entitled "Some Effects of a Virile Chemical Industry on Food Processing", discusses in one section the use of quaternary ammonium compounds for sterilising, and anionic wetting agents for cleaning in the food industries; some results having a bearing on toxicity are included, and these deal with the haemolysis of blood by a germicidal quaternary ammonium compound, a sodium alkyl sulphate, saponins and soap, the first three named being tested in 1:10,000, 1:50,000 and 1:100,000 parts of water. With the quaternary ammonium compound complete haemolysis in the test is obtained with a dilution of 1 part in 50,000, but with the sodium alkyl sulphate, 1 part in 50,000 gives only partial haemolysis, while complete haemolysis necessitates the higher concentration of 1 part in 10,000.

Since the quaternary ammonium compounds are used in dilutions usually of 1 part in 5,000 and sometimes in even lower concentrations, and solutions of wetting agents such as sodium alkyl sulphates often in concentrations of anything between 0·1% and 1%, it could be stated from this test that the use of the latter class of substances should be carefully controlled, although the results also show that the sodium alkyl sulphate is much more easily rinsed from the surface than the quaternary. On the other hand, from the actual concentrations published, it is evident that this haemolytic effect is greater with the quaternary ammonium compound than with the sodium alkyl sulphate, weight for weight.

The ease with which quaternary ammonium compounds are adsorbed on to surfaces and organic matter generally, in the latter case often resulting in complete inactivation, appears to be a good reason for assuming that minute traces which are likely to come in contact with food cannot have any effect in the system. The question of the lysing of blood would not enter into it, unless there was a lesion producing internal haemorrhage and sufficient uninactivated material was present. Whether these substances can enter the bloodstream through healthy tissues also requires investigation before the value of such results can be assessed.

The question of toxicity is discussed in Chapter III, but the author has been prompted to make these few remarks because they show that laboratory experiments, such as the one quoted above, can be evaluated in two different ways, and caution is necessary in the interpretation of such experiments.

Certainly all possible evidence must be collated on toxicity and considered impartially by some competent independent body, and the investigation should not be confined to new substances whose potentialities are still the subject of investigation, but also to a very long list of accepted materials with which the human body comes in contact either in industry or in foods.

PART I

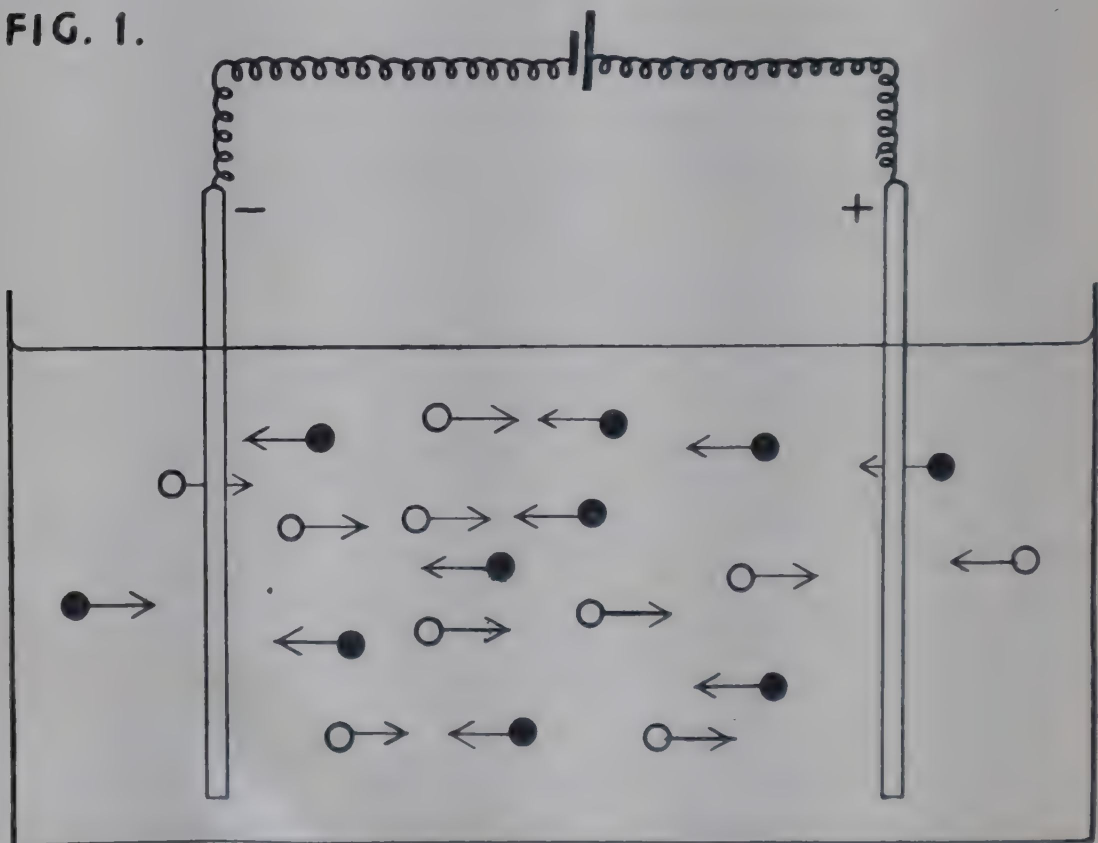
PHYSICS, CHEMISTRY AND BACTERIOLOGY

CHAPTER II

PHYSICAL PROPERTIES OF SURFACE-ACTIVE COMPOUNDS GENERALLY

THE germicidal quaternary ammonium compounds belong to a general class of organic substances which are described as being surface active. When they are dissolved in water, some proportion of the quantity dissolved will ionize, giving anions and cations, and in this case it is the cation which is significant, and for this reason they are often referred to as "cation active".

FIG. 1.



Simple diagram of anions O→ and cations ←● in the Electrolysis of a solution of sodium chloride, Na Cl.

Na^+ ←● cations moving towards − electrode.

Cl^- O→ anions moving towards + electrode.

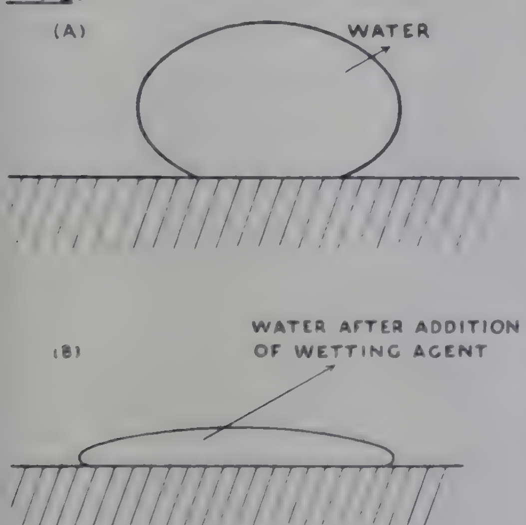
It may be useful at this point to give a definition of a surface-active compound. When a surface-active compound is dissolved in water, the latter is found to spread more readily on greasy surfaces, to be slippery to the touch and very often to foam easily on shaking. Scientifically, a substance may be described as surface active if, when in solution, it manifests itself by imparting to the surface, and other boundaries or interfaces, properties which reflect its structure and composition, rather than the normal characteristics of the solvent in which it is dissolved.

There are a great number of substances in everyday commercial and domestic use which belong to this class of compound. The best and most widely known are the soaps, and after these come the new synthetic detergents, or, as they are sometimes called, "wetting agents", of which commercial products such as Teepol, Iranopol and Perminal are representatives. The soaps and these wetting agents all dissolve in water giving surface-active anions or, as mentioned above, the important part of the molecule resides in the anion, the anion being that ion which, when in solution, is attracted to the positive pole of an electrolytic cell when current is passed. (Fig. 1.) Hence the anion bears the negative sign, and the cation the positive.

A further class of surface-active compound is an organic molecule resembling in general organic construction, both the anion-active and the cation-active compounds, but which does not ionize in solution and is, therefore, known as "non-ionic". Nevertheless, the essential characteristics which produce surface activity are present in this type of molecule just as well as in the type which ionizes.

The most obvious characteristic of a solution which contains a surface-active compound, at least of the types which have been mentioned above, is the ease with which such a solution spreads over a greasy surface ensuring good wetting of the surface, very often foaming quite easily. A simple experiment demonstrates this very well. If a little water is spilt, for example, on a polished wooden surface or a greasy metal one, it will be seen to remain as globules or patches, the edges of which are quite steep with respect to the metal surface. (Fig. 2(A).)

FIG. 2.



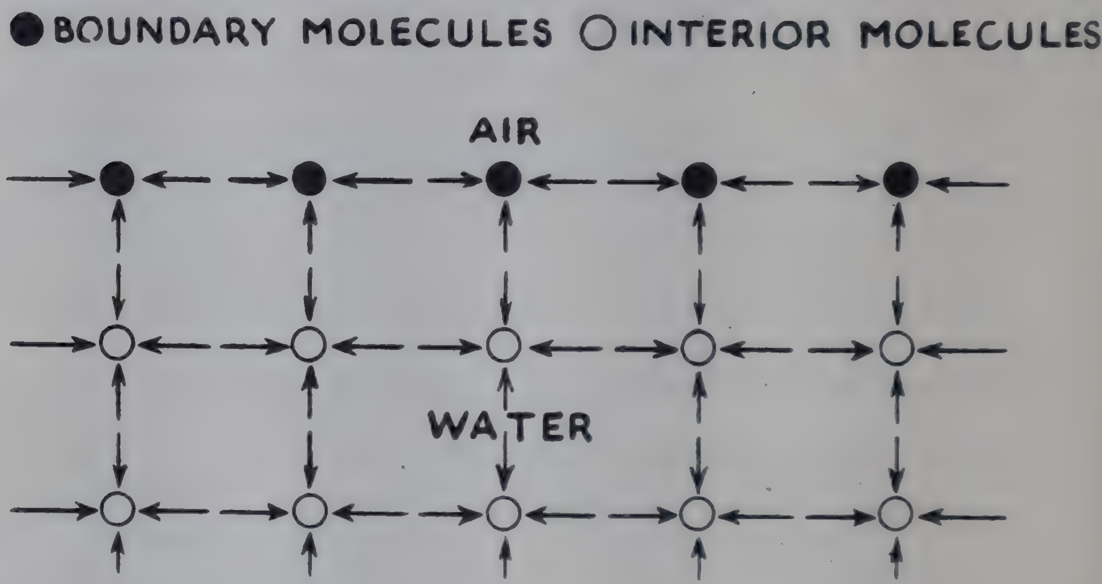
If a small trace of a solution of a surface-active compound is then added, the globule will at once be seen to flatten out and spread over a greater area, indicating that the addition of the wetting agent has greatly improved its power of wetting and spreading. (Fig. 2(B).)

Such solutions are slippery or soapy to the touch, and on being shaken in a tube will soon produce a considerable head of foam. The fact that the globule of water showed better ability to spread on the greasy surface, was due to the surface tension of the water being lowered, and all soluble surface-active compounds have the power to lower the surface tension of water to a greater or less degree.

If we consider for a moment a single drop of water, we shall be able to obtain a picture of surface tension which will be a useful preliminary to an understanding of how surface-active compounds

FIG 3.

SMALL SECTION OF A DROP OF WATER SHOWING HOW ATTRACTIVE (COHESIVE) FORCES AFFECT THE BOUNDARY MOLECULES



work. Obviously a spot of water is a conglomeration of millions of water molecules, and each molecule of water is exerting an attraction on its neighbours; this in fact produces the necessary cohesion which keeps the molecules together and enables them to form drops.

In Fig. 3 it is seen that whereas molecules inside the drop are being attracted by their fellows on all four sides, the molecules in the surface have attraction on only three sides, so that these boundary molecules are under forces of attraction which naturally are directed towards the interior of the drop which results in a spherical shape. This is true, of course, for all liquids since the same rule will apply to petrol or sulphuric acid as readily as it will to water. The important difference comes when we have to consider that the intensity of the forces of attraction among the molecules varies from liquid to liquid; and the greater attraction between the molecules, the greater will be the force pulling the boundary molecules

into spherical form, and the more readily will they assume that spherical form and maintain it. In other words, there will be a pull on the surface or boundary molecules which varies directly as the intensity of the intermolecular forces, so it will seem as if there is a kind of skin on the outside of the drop, and this "skin" is in fact the direct cause of surface tension. Therefore, the greater the molecular attraction in a liquid, the greater will be the surface tension.

Generally speaking, organic liquids which consist very largely of carbon atoms united to hydrogen and occasionally oxygen, have rather low intermolecular powers of attraction which results in a relatively low surface tension; but inorganic liquids containing as they often do, more powerfully attractive and reactive groups of atoms, are often higher, while highest of all come metals in the liquid state. For example, mercury which is always a liquid at ordinary temperatures, has an extremely high surface tension, and manifests this in its persistence to assume globular form very easily.

Surface tension is measured in dynes per centimetre, dynes being the unit of force and a linear measure of one centimetre along a straight line being the convenient convention.

Water has a fairly high surface tension because the powerfully attractive oxygen atom has only two hydrogen atoms attached to it, so that the residual forces are quite high, in other words the proportion of powerfully attractive atoms to the feebly attractive is relatively large.

The following table gives the surface tension of a number of liquids measured at ordinary room temperature, and will illustrate the variations which may be encountered.

TABLE I

SURFACE TENSIONS OF SOME LIQUIDS

	dynes/cm.
Ethyl alcohol	22·3
Benzene	28·8
Benzophenone	45·1
Water	73·0
Mercury	465·0

Water has been used for ages as a cleaning agent, but one of its greatest limitations is due to its high surface tension, hence the need of soaps, etc., to lower this in order to facilitate spreading and wetting in the cleaning process as well as the other functions of emulsification, etc. Many processes of disinfection also depend upon the lowering of the surface tension of the aqueous solution, in order to ensure better penetration of bacterial films and crevices into which ordinary water could not penetrate because of its high surface tension.

It is now possible to proceed to consider how surface-active compounds achieve this reduction of surface tension, and firstly it is necessary to appreciate the two essential component parts of a surface-active compound. The insolubility of oils of all kinds in

water is something which needs no explanation; by oils are meant greases, paraffins and anything of an essentially hydrocarbon nature. These oils consist of chains or rings of carbon atoms, each carbon atom having one or more hydrogen atom attached to it, according to the type of compound. (Fig. 4 (A).)

FIG. 4

(A) $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$

NORMAL OCTANE: A HYDROCARBON CHAIN CONTAINING EIGHT CARBON ATOMS WITH HYDROGEN ATOMS ATTACHED, IMMISCIBLE WITH WATER AND WATER REPELLANT.

The possible number of compounds of this type is almost infinite, and normally these substances, although often inflammable in the gaseous state when mixed with air or oxygen, show surprisingly little chemical activity in other directions. If, however, by chemical means a compound is produced which consists of such a chain of carbon atoms with a chemically reactive group attached to it, then we have present the two essentials for a surface-active compound. This reactive group is often acidic in nature, and on being neutralised forms something of the type of a soap or anionic wetting agent. (Fig. 4 (B).)

(B) $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2O \cdot SO_2ONa$

SODIUM OCTYL SULPHATE A SIMPLE WETTING AGENT

THE $-O \cdot SO_2ONa$ GROUP IS POWERFULLY WATER ATTRACTING

The compound has now two parts, one the oily water insoluble carbon chain part, and the other the acid salt group which has the tendency to be very soluble in water, so that there are now two opposite tendencies combined together in the one compound. If a small quantity of such a substance is dissolved in water, a struggle between these two properties at once takes place, the oily chain, as it were, trying to pull the molecule out of solution, and the water-attracting sodium salt part trying to keep it in. The net result of this conflict is a compromise whereby the molecules, which may or may not be ionized, are attracted to the boundaries of the solution where they form a layer overlying the water molecules, and impart to the surface or boundaries of the solution some of the properties of an oil which involves, of course, the property of a low surface tension. This phenomenon is called "adsorption" or the migration of the molecules to interfaces. (Fig. 5.)

As soon as a fresh surface or boundary or interface is created, at once the adsorption phenomenon begins to take place, so that the insertion of a piece of metal into a beaker which already has an air-solution interface and a glass-solution interface, results in the creation of a new film of surface-active molecules at the metal-solution interface. (Fig. 6.)

FIG. 5.

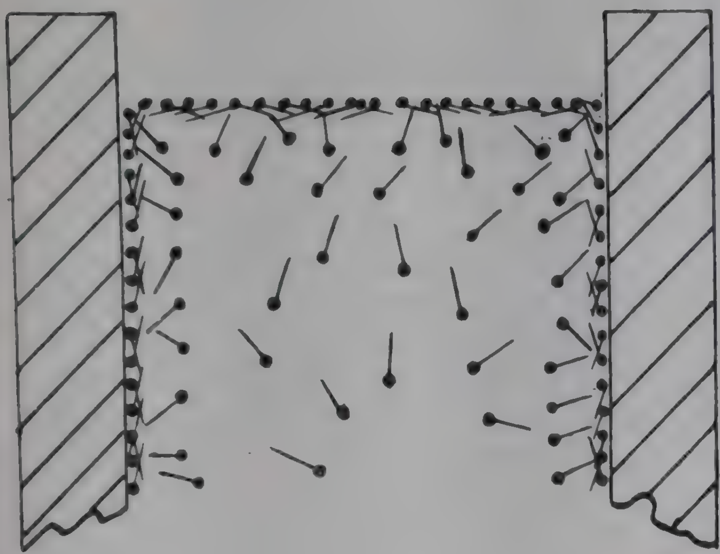
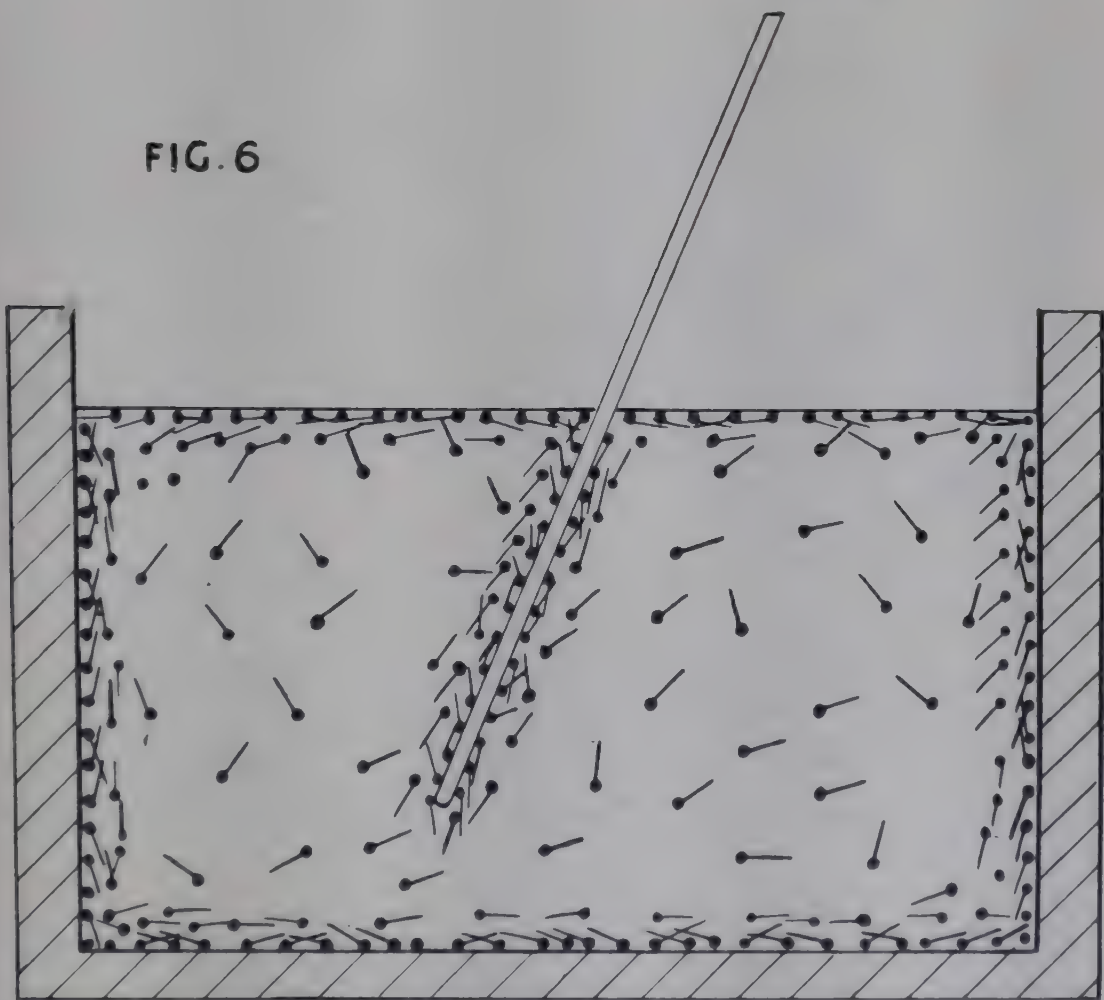


FIG. 6

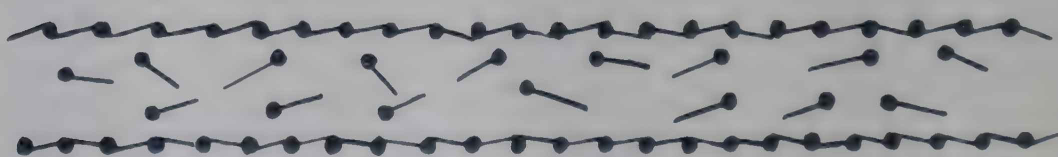


Creation of a new interface by the insertion of a strip of metal into a surface active solution.

Naturally, if sufficient surface-active material is dissolved in the water, there will also be a considerable number of molecules still in the bulk of the solution, but it is possible, by so regulating the amount or dilution of the surface-active agent, to have most of the molecules concentrated at the interfaces and only very few in the bulk of the solution. This point will be referred to later on when discussing the practical applications of quaternary ammonium compounds.

All the foregoing descriptions and remarks apply just as well to the cationic surface-active compounds to which the quaternary ammonium series belongs, as they do also to the non-ionic wetting agents. The rate of adsorption is also quite important and varies for each different substance, some adsorbing rapidly to the interfaces and others more slowly. Generally speaking, the rule for a soluble surface-active compound is that the greater the proportion of the hydrophobic part of the molecule, the more rapidly will the migration from the interior of the solution to the boundaries take place.

The question of the foaming power of these surface-active compounds is closely related to the rate of adsorption. It must be borne in mind that the question of low surface tension is not directly related to foam stability, and further that foam formation and foam stability are two different things. Violent agitation will cause any liquid to entrap bubbles of air, but the film forming these bubbles breaks very rapidly upon the cessation of agitation, and the foam has a very short life. The presence of a surface-active agent in water usually tends to impart to the bubble film elasticity, and in the bubble film there are layers of the surface-active molecules adsorbed to both the outer and inner boundaries of the film. Taking, for example, the question of a soap bubble of about $\frac{1}{4}''$ in diameter, we may visualise the adsorption in that film to be on the lines shown diagrammatically in Fig. 7 (A).



(a) Section of film of Stable Bubble: heavy adsorbed films and molecules in between which can adsorb on expansion.

FIG. 7 (A)

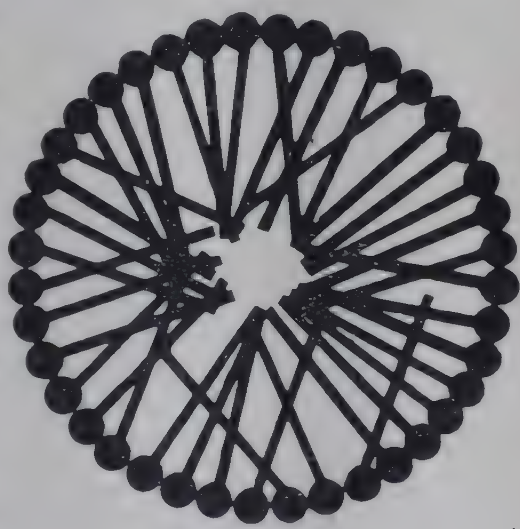
Here we have an upper boundary of adsorbed molecules and a lower boundary of adsorbed molecules, and between the two water which also contains some molecules of the soap. If the bubble is now expanded to three or four times its size, we shall see in Fig. 7B that the area of the film has been greatly expanded, the thickness of the

(b) Section of same film after expansion: adsorbed films stretched to limit. FIG. 7 (B)

film considerably reduced and a greater number of surface-active molecules has been attracted into the boundary by adsorption. If the speed at which the bubble is enlarged is not too great for fresh molecules to go to the boundaries, then the bubble will be quite stable; but if it is expanded at a speed which does not give time for fresh molecules to be adsorbed, then the elasticity of the film will be so weakened that the bubble will burst because the adsorbed surface-active molecules will not form a continuous film.

If instead of soap we have a surface-active molecule with a slow rate of adsorption, then any variation in the size of the bubble may well bring about its collapse. It must be mentioned that the strength and elasticity of a bubble film depends upon some ancillary factors such as the drainage rate of the bubble, viscosity of the solution which can be sometimes improved by the addition of non-surface-active materials, and also the configuration of the surface-active molecule itself.

FIG. 8.



Ionic Micelle

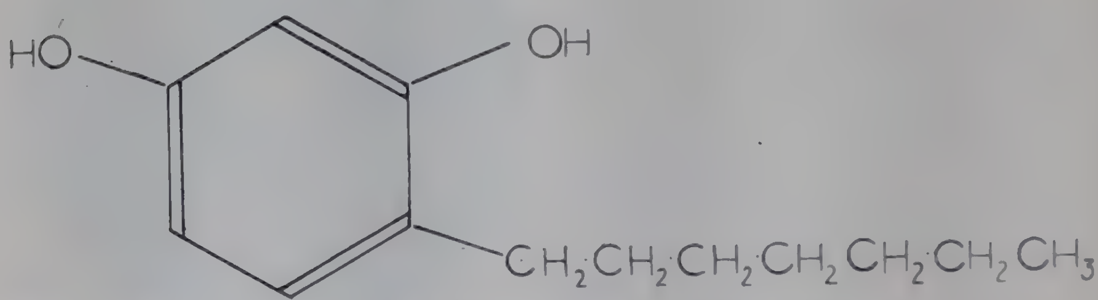
There is also another interesting property which surface-active compounds have in solution. That is that they have a tendency to form ionic micelles which are aggregations of the surface-active ions, and this will take place at a certain critical concentration for each compound. These ionic micelles are spherical in shape, and the water attracting part of the ion lies on the periphery of the sphere and the long fatty chains are directed towards the centre as depicted diagrammatically in Fig. 8.

The micelles formed from soap have been known for a very long time and quite carefully studied. It has since been recognized that all other surface-active materials tend to do the same kind of thing, at any rate those which ionize. Inside the micelles there is quite a considerable amount of unoccupied space which can be filled up by other substances; for example, an ionic micelle can be made to entrap oil or liquid impurities and hold them securely, while superficially the solution appears to be clean. On dilution with water to

the point at which the ionic micelle breaks down, opacity appears as the insoluble oil or impurity is liberated into the solution. Sometimes this creates a problem in the purification of certain quaternary ammonium compounds.

The significance of physico-chemical properties in bactericides and disinfectants has long been recognized in another series of compounds, the phenols. Taking resorcinol, which is a dihydric phenol, as an example, we find that the surface tension of a 0·01% solution is 76 dynes per cm. which is actually a little higher than water, but when a propyl group is introduced into the molecule, 4-propyl resorcinol in the same concentration, has a surface tension of 73. The next one, 4-butyl resorcinol shows a surface tension of 66 and so on with the lengthening alkyl chain; 4-amyl resorcinol has a surface tension of 60 in a 0·01% solution, 4-hexyl resorcinol 54 and 4-heptyl resorcinol 43. When the activity of these compounds in aqueous solution against *S. aureus* and *E. typhi* organisms are considered, it is seen that heptyl resorcinol has greater killing power than any of the others, although the killing power of all the members of this series increases steadily from resorcinol to the 4-heptyl derivative. (Fig. 9.)

FIG. 9.

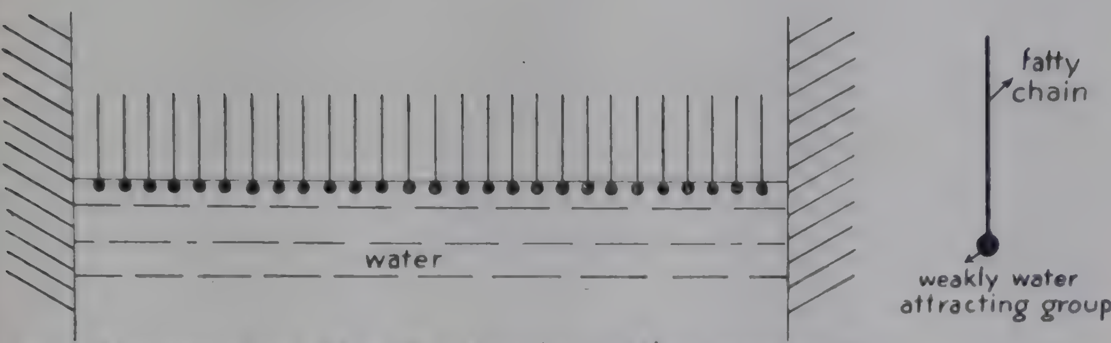


A surface active phenolic germicide. 4-heptylresorcinol

A brief survey has now been made of the more important physical properties of surface-active substances, and it will be seen in other chapters how these properties are responsible for many of the phenomena observed during their practical usage.

One further point may be made with regard to the adsorbed molecules or ions which makes the solution so obviously different from the water in which they are dissolved. The fatty chains in this film may be regarded as laying flat in the surface, and not at an angle which is the way they are sometimes represented as doing. The type of molecule which does form a film with the chain standing upright from the surface of the liquid, is represented by such compounds as oleic acid where the acidic group is weakly water attracting, and forms a kind of anchor with the fatty chain almost at right angles to the surface and packed closely with the neighbouring molecules. (Fig. 10.)

FIG. 10.



Insoluble adsorbed film of molecules with a weak water attracting group e.g. oleic acid.

It has already been mentioned that surface-active compounds, when in solution, are adsorbed to the surface or other boundaries of the solution. The nature of the substance forming the boundary of the solution, may play a part in determining the behaviour of the adsorbed surface-active molecule when it reaches the boundary.

It is a fact that the cationic quaternary ammonium compounds are more strongly adsorbed on to metals and glass than are the anionic substances of the soap or wetting agent type. The fact that these compounds can attach themselves strongly to surfaces, has an important influence on their usefulness as anti-bacterial agents.

It is quite easy to demonstrate this strength of adsorption by taking a clean piece of glass which has, for preference, been cleaned in concentrated sulphuric acid to which sodium dichromate has been added. Afterwards the glass is rinsed clean in pure distilled water. Glass cleaned like this is as near to being absolutely clean as it is possible to get it, and it will be noticed that ordinary distilled water spreads over this glass perfectly smoothly and without any trouble, showing that it is free from any film of grease. If this piece of glass is now immersed in a solution of a quaternary ammonium compound for a few moments, and then withdrawn and rinsed again with pure distilled water, it will be noted that there is apparent an invisible film of grease present which results in the water shrinking into globules and patches on the surface. This film of the adsorbed quaternary compound is so minute as to have little practical significance, and yet by means of this simple test its presence can be demonstrated.

To summarise the contents of this chapter, it can be said that germicidal quaternary ammonium compounds belong to the general class of surface-active agents. Surface-active agents are of three kinds—anionic, cationic and non-ionic, but all have a water attracting portion and a water repelling carbon chain or organic grouping. When dissolved in water, surface-active substances manifest themselves at the boundaries or surface of the solution, bringing about a reduction in the surface tension of water which makes

for good wetting power, spreading and penetration. In many cases, the manifestation also includes a considerable degree of foaming power, but this does not always, and need not necessarily, follow. The property of the surface-active molecule in forming an interfacial or boundary film, is called "adsorption" which should not be confused with "absorption". The quaternary ammonium germicides differ from anionic compounds such as soaps and synthetic detergents, by carrying the opposite charge on that part of the molecule which is responsible for the surface-active properties. They differ also in being much more strongly adsorbed to metal, glass and other surfaces and having powerful anti-bacterial action, whereas that of the anionic compounds is generally weaker. In the more concentrated solutions, the quaternary ammonium germicides will form ionic micelles, and this is similar to the behaviour of the anionic substances.

CHAPTER III

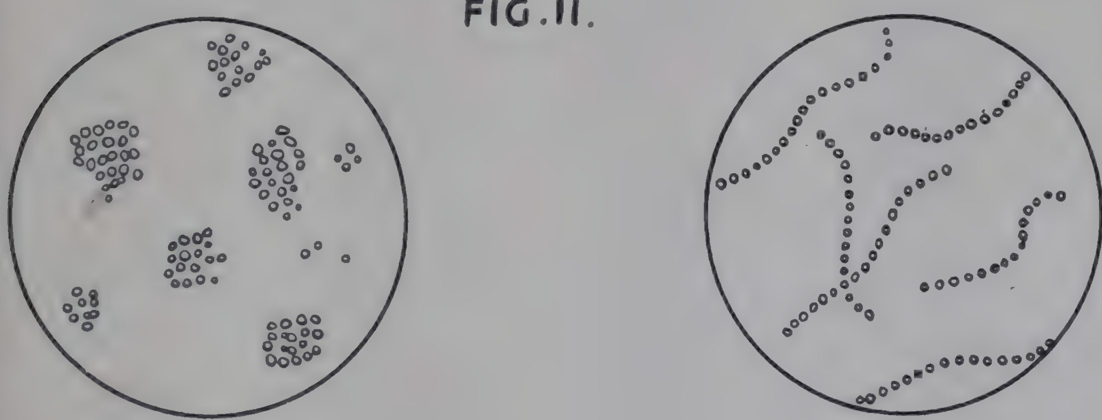
ANTI-BACTERIAL ACTIVITY AND TOXICITY

MICRO-ORGANISMS generally can be considered in four classes: those which are dangerous to health (pathogens), those which can spoil foods and beverages, those which are definitely useful such as yeasts and also certain bacteria, and a great number outside these groups which are neither harmful nor useful, although some of these classes overlap. In this field nature resists extermination by a variety of means, and these means often result in the production of various forms of organisms and adaptations which enable them to overcome an otherwise unsatisfactory environment.

Kinds of Micro-organisms

The micro-organisms against which the quaternary ammonium compounds are used are viruses, bacteria, yeasts, fungi (moulds) and protozoa. Viruses are extremely minute forms of life, and

FIG. II.



Staphylococci   Streptococci

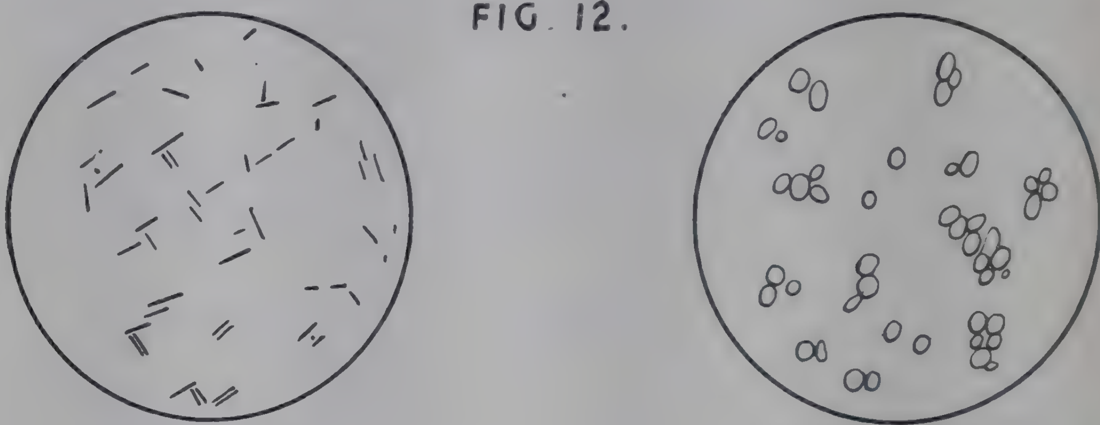
are thought to be large complex protein molecules often with a molecular weight of several millions. They are so small that methods used for the isolation of bacteria are wholly inadequate for viruses. Some infectious diseases are caused by them, but much research work has yet to be done in this field.

The lower bacteria consist of exceedingly small organisms which are single cells, being, in fact, minute masses of protoplasm contained in a membrane. They increase by methods such as simple fission, and in size they vary between 1/1000th and 5/1000ths of a millimetre in length. They may be spherical, as are the micrococci, or they may be rod-shaped, as in the case of the test organisms *Esch. coli* and *E. typhi*, or else having a wavy or spiral appearance such as the spirilla. Among the spherical forms, staphylococci and streptococci

are some of the best known. Staphylococci tend to grow in groups like bunches of grapes, and streptococci form chains of various lengths. (Fig. 11.)

A representative of the class of staphylococci which receives much attention in medicine is *Staphylococcus aureus*, which is usually responsible for the common boil. Several varieties of streptococcus are responsible for many diseases, including sore throats, but it must be borne in mind that there are a great number of members of all these main families of organisms. The rod-shaped cells are themselves divided into two groups, firstly *Bacterium* properly called, which does not form spores, and secondly the *Bacillus*, which does. (Fig. 12.)

FIG. 12.



Escherichia coli — S. cerevisiae (yeasts)

Even the most elementary discussion of bacteria would be incomplete without reference to the significance of coliform organisms. *Esch. coli* is a microbe found extensively in intestinal flora. So long that it remains in the intestines it is a useful type of bacterium, although occasionally in the human body it gains access to organs where it can cause trouble. In food testing, water bacteriology and sanitary work generally, it has a particular significance in that, when this organism is detected, it means that there is a possibility of faecal contamination. In other words, that contamination of the intestines of the animals is present. The significance of this observation needs no further discussion. The detection of coliform organisms means that other related types causing serious diseases such as typhoid fever and dysentery, may also be present, and therefore the coliform test is used as an indicator test. It does not mean that because coliform organisms are present in any bacteriological examination, the dangerous disease-causing germs are also there, but it does mean that the possibility of that type of infection cannot be ruled out.

The coliform test is carried out by the inoculation of the sample to be tested into a particular type of medium called "MacConkey's Broth", when, after incubation at 37° C. for 48 hours, gas is evolved

and this is collected in the tube in a small inverted tube, which at the beginning of the test is filled with the broth, but the broth in the little tube is displaced by the gas, and the clear evidence of gas formation is obtained.

This, in brief, is an outline of the coliform test, although various modifications have been introduced. It is usually carried out side by side with the normal plating and examination for other types of bacteria, and it is commonly used in all examinations carried out in dairies, ice cream, and many other food plants, as well as in the examination of waters which are intended for human consumption. For this reason it has been found convenient to make *Esch. coli* a test organism for quaternary ammonium compounds. Fortunately, *Esch. coli* is more resistant to disinfectants than are the germs which cause typhoid and dysentery and other diseases, which are often encountered in food-poisoning epidemics. The significance of a positive coliform reaction in bacteriological testing, as described later on in this book, will be apparent to the reader.

The higher bacteria form very long chains and can often be seen as interwoven masses of filaments. They show signs of greater organization and development than the lower bacteria and are often very specialised in their habitat and functions.

Next to be considered are the yeasts. Yeasts (Fig. 12) are usually round or oval organisms bigger than the lower bacteria, although they are uni-cellular in the same way. They decompose sugar solutions into alcohol and carbon dioxide which is, of course, the fundamental principle of brewing and wine making. They multiply by budding off from the main yeast cell, a smaller cell which eventually separates and leads an independent existence. Yeasts are very abundant in nature; there are at least 500 known varieties. The diameter of a yeast cell usually is about 1/100th of a millimetre. Yeasts are not considered as a problem to health, but can be a serious menace to the keeping qualities of many foods and beverages, although being extremely useful when controlled for the specific function of fermentation. Very few harmful yeasts are known, and from this point of view they can, perhaps, be ignored.

Moulds are a primitive form of plant life and more highly developed than the bacteria and yeasts. They differ very considerably from the simple uni-cellular bacteria and yeasts, and consist of roughly three parts which parallel the roots, stem and foliage of the higher plants.

The Cell Wall: Staining with Dyestuffs

The bacterial cell can be considered as consisting of protoplasm surrounded by a cell wall. Through the membrane-like wall the cell must take its food and excrete waste products. That the nature of the cell wall of bacteria shows considerable chemical differences between different species is now well established, and chemical variations in the wall are undoubtedly responsible for the different

kinds of staining effects which may be produced, and also for variations in resistance to chemical disinfectants such as quaternary ammonium compounds.

Bacteria, being generally colourless and minute, would be invisible even under the microscope, except for the device of staining with dyes. The results of staining bacteria with various dyes are quite important and have led in one case to a rough classification of the majority of bacteria, dividing them into two groups. With this particular staining procedure, which is known as Gram staining, certain organisms appear under the microscope, after the staining treatment, as a deep purple-black, whereas other types appear pink in colour. The former class are called Gram-positive and the latter, which are pink, are known as Gram-negative types.

This difference in reaction to Gram staining reflects fundamental differences which have been observed with micro-organisms, and seems to indicate a considerable chemical difference in the cell wall; not only perhaps a chemical difference, but a physical difference as well, and this is often reflected in the reactivity of the germs to chemical disinfection. Whereas a great number of chemical reagents can be used to destroy or prevent the growth of Gram-positive organisms, very few are found to have the same degree of activity against the Gram-negative types. This is generally true for the quaternary ammonium compounds, because in common with the great majority of chemical agents, they are far more active against Gram-positive than against the Gram-negative types. Practically all reported tests by the usual methods of anti-bacterial testing show that much higher concentrations of the germicides are needed to kill the Gram-negative than the Gram-positive organisms.

A brief description of the Gram staining method may be useful. Many variations of this have been produced, but one method consists of staining the film with methyl violet solution for 15 seconds, and then washing off this dye with an iodine solution consisting of one part of iodine, three parts of potassium iodide and 300 parts of water. The slide is then flooded with the iodine solution for 30 seconds. The excess of iodine is then poured off and the slide washed with alcohol until no more colour comes away from the film. When the alcohol, which is running off, is quite colourless, the washing can be considered at an end. A wash in pure distilled water follows, and then counter-staining is carried out; and for this purpose a $\frac{1}{2}\%$ solution of safranin in water for one minute is quite satisfactory, and it is impossible to overstain by using this dye.

It has been suggested by some workers that the difference in the Gram stain reaction to different organisms is not so much qualitative as quantitative, that is to say that the Gram-positive organisms adsorb or absorb a greater quantity of the dye, whereas the Gram-negative takes so very little that it appears merely as a pink colour. Some people have suggested that the Gram-negative cell wall has a harder, more compact and less porous texture to

account for this difference in degree, but other workers are of the opinion that the difference in staining is due to different chemical constituents of the cell wall which do not receive the dye molecule in the same way, or as heavily. Whether this is so or not, it is certain that the Gram staining procedure is a very important one, not only from the point of view of the general classification of bacteria, but from the reflection of the susceptibility of these organisms to chemical disinfectants.

Spores

We have already briefly referred to another interesting classification which is provided by the names *Bacterium* and *Bacillus*. Both of these are rod-shaped micro-organisms, but *Bacillus* differs from *Bacterium* in this classification because of its ability to form spores, whereas *Bacterium* is unable to do so.

In the lower bacteria, and in this case we use the word "bacteria" as the general name for the lower micro-organisms rather than as a specific one as above, the ability to form spores belongs only to this class of micro-organism, and to the related *Clostridium*.

Spores may be considered as a resting condition of the organism when it finds itself in an environment inimical to growth and development. Certain factors may stimulate spore formation, such as lack of food or moisture or high temperatures, and then the normal cell disappears and small granules or minute spheres take its place. The organism is now in a resting condition and it can withstand heat, chemical disinfection and the absence of food and moisture to an enormously greater degree than could the ordinary rod-shaped cell. The ordinary cell is known as the vegetative organism to differentiate it from its resting condition or spore. Spore-forming organisms provide very serious problems in the food industries because of their resistance to destruction.

Inside the bacterial cell itself there is a fluid mass of protoplasm which contains various kinds of substances, some simple inorganic salts and more complex organic proteins. Granules of one sort or another have also been observed in the protoplasm, and knowledge of the functions of the various constituents is, of course, the subject of an ever-increasing range of research work.

Thus, beginning at the lowest end of the life-scale, there are viruses and then bacteria, yeasts, moulds and microalgae on the plant side and also protozoa on the other hand which may be regarded as the most minute form of animal life.

Protozoa are also masses of protoplasm surrounded by a membrane, but these cells contain well recognised and definite nuclei. Several forms of protozoa produce disease in men and animals, and some of these have been the subject of attack by quaternary ammonium disinfectants with almost the same degree of success which has been recorded against bacteria, yeasts and moulds.

Omitting the viruses for the time being as an unknown quantity,

a generalisation can be risked by saying that of the bacteria, Gram-positive organisms are more readily destroyed by quaternary ammonium germicides than are Gram-negative bacteria. Yeasts are generally rather more resistant than the Gram-negative organisms, while moulds appear, in some cases, to be more resistant than yeasts.

Mode of Action of Disinfectants

When considering the question of the way in which different kinds of antiseptics and disinfectants act upon micro-organisms, it is necessary to proceed with care, and the many-sidedness of truth is well demonstrated by the confusing and often opposing views expressed, with relation to the chemical and physico-chemical reactions of the bacterial cell.

It seems clear that there are different kinds of anti-bacterial action. Some disinfectants appear to act by interfering with the nutrition and respiration of the micro-organisms, and others by means of a more violent and destructive chemical action on the protoplasm, as for example chlorine, hydrogen peroxide and other oxidising agents. Another mode of action is the de-naturing of the protein constituents of the cell such as by heat and the heavy metals, while surface-active agents appear to cause a disintegration of the cell, which is probably due to physical action following the adsorption of the molecule on the outside membrane or wall.

Other compounds appear to act on particular parts of the cell; for example, many well-known dyestuffs are found to have anti-bacterial action, and it is assumed that they replace similarly charged ions on the cell which are necessary for the normal functioning of the bacteria, but when these normal ions are removed and replaced by an ion of the dyestuff, the metabolism and general life of the cell is so seriously interfered with that it dies.

Dyestuffs can be both anionic and cationic, and, it is interesting to note, are invariably rather large molecules. Phenols probably act in the same way. They are found to be most effective when in acid solution, but comparatively useless once the phenol has been made into its sodium salt by means of alkali, no interaction taking place between the sodium salt of the phenol and the surface of the cell, since the ability of the phenol to form any kind of link with a basic group is now effectively prevented.

It is assumed that the phenols form an un-ionized complex on the bacterial cell, and by this means interfere with its normal growth and metabolism. The phenols are also slightly surface active, and as was discussed in Chapter II, they can be made considerably surface active by the introduction of carbon chains of increasing length, so that in the case of the resorcinol series, hexyl resorcinol has considerable surface activity and is amongst the most powerful of anti-bacterial phenols known. In fact, the formation of un-ionized complexes by molecules of large molecular

weight and surface activity provides an important if not the most important basic condition for chemical disinfection. Obviously the quaternary ammonium compounds belong to this latter class of disinfectants, and it seems very likely that when they are adsorbed on the cell wall, they form un-ionized complexes which are responsible for the death of the cell. It must be admitted, however, that there are many things as yet undecided about the exact mode of action.

Bacteriostasis

A most important phenomenon in connection with the action of many chemical disinfectants, and one which affects the quaternary ammonium class most particularly, must now be considered.

It may be said that anti-bacterial action of any chemical belongs to one of two kinds, the two kinds being bacteriostatic and bactericidal activity. The meaning of the term "bactericidal" is, of course, obvious; it refers to the complete death of the micro-organism. Bacteriostasis, on the other hand, means that the germ or micro-organism is prevented from growing and multiplying. Thus a single bacterial cell, when treated in such a way as to bring about bacteriostasis, will not necessarily die but will simply fail to multiply, and so instead of one organism becoming several million in a few hours, it will merely remain as one single cell. It is unfortunate that in these descriptions a number of reservations have to be made. So it is with bacteriostasis because the latter statement will only hold good so long as no other secondary changes occur in the organism which may themselves bring about its death.

Superficially, a bacteriostatic effect may be mistaken for bactericidal action if no special precautions are taken to distinguish one from the other. The usual means of testing any kind of disinfectant consists, in principle, of taking a suspension of the germs, sometimes several millions, which may all be contained on one cubic centimetre of bacterial culture, and adding to this suspension a certain volume, perhaps 4 or 9 c.cs. of the chemical disinfectant which is in solution at a certain strength. In the case of the quaternary ammonium compounds, it may be only 1 part of quaternary in 5,000 or 10,000 parts of water.

The mixture of disinfectant solution and the microbes are then allowed to remain together for a definite period of time. This again is arbitrarily chosen and may be five, ten or fifteen minutes, or even periods very much less, according to the information which the investigator wishes to obtain. After this time, a small proportion of the mixture is taken and added to a suitable solid nutrient medium in a Petri dish, which encourages the growth of that particular class of micro-organism. This Petri dish is then placed in an incubator and allowed to remain usually for 48 hours at the best known temperature for causing that particular germ to grow, so that it has plenty of the right food and just the right temperature

to encourage rapid growth. If, after 48 hours, this Petri dish is examined, one of two things will be seen—either the dish will be quite clear since the agar which is usually used for most cases is almost transparent, or else it will be seen to contain a number of white spots or spots of some colour—some may be yellow or some may be green, according to the organism. These small spots will be colonies of the germs, and each colony will have grown from one single cell which was taken out of the medication tube where the bacteria and disinfectant were mixed. If the plate is clear, then it can be assumed at any rate, that no living microbes were transferred from the medication tube. *But note*, this is only an assumption. If, on the other hand, the plate contains some number of colonies, it is evident that the disinfectant did not kill all the germs in the medication tube.

The foregoing is just a brief description of a general method which is modified in many ways in actual practice, but the point is, that even if the plate is absolutely clear, it does not mean that because no colonies have grown all the organisms are dead, because bacteriostasis may be merely causing a number of organisms to remain quiescent and not to develop. Thus, a simple method of this type cannot distinguish between bactericidal activity and bacteriostasis, and it is important, if the real killing power of any disinfectant is to be known, to be able by some means to distinguish the one from the other.

Bacteriostatic power is exhibited, as previously observed, by a large number of chemical substances, some of which are bactericidal when used in a sufficiently high concentration and only behave as bacteriostatic agents when they are much more dilute.

It has been found that by treating the contents of the medication tube with some substance which neutralises the disinfectant, its effect on the organism can be modified, and when a sample from the tube is removed to be plated, colonies may be found to develop more readily.

As an instance of de-toxication, the behaviour of a culture of *S. aureus*, after being exposed for two hours to a 1% solution of mercuric chloride, may be quoted. After this time the contents of the tube were treated with certain sulphur compounds which reacted with the mercuric ion, precipitating an insoluble mercuric compound. This resulted in the removal of the mercuric ion from the bacterial cell, and the cell was able to resume its normal functions and growth. This demonstrated that, up to this time and with the particular organism used, the effect of the mercuric chloride had been purely bacteriostatic.

Germs which have been in contact with quaternary ammonium solutions, have been revived after the cells have been treated with either an anionic wetting agent or else with a class of substances known as phospholipids, although the treatment of the cells with the de-toxicating compound must be carried out within a short time

after their exposure to the quaternary ammonium compound, usually within 15 to 20 minutes. Valko and DuBois[1] came to this conclusion, but other workers, for example Klein and Kardon,[2] were of the opinion that the anion apparently only serves to save the organisms not already killed by neutralising the remaining quaternary. In fact, if the effect of a disinfectant can be reversed by the treatment of the germs with some convenient de-toxicating agent, then bacteriostasis would appear to be the real effect of that dilution of the germicide.

It is, however, necessary to give a warning against pitfalls in this kind of work, because different microbes will show different powers of resistance to germicides, and the strength of the solution and the length of time of exposure are also important.

In the field of the quaternary ammonium compounds, it appears that the anti-bacterial effect is dependent upon, firstly, the concentration of quaternary used, and secondly, the length of time the organism is exposed to the quaternary solution, and that the bacteriostatic effect is quantitative rather than qualitative. Whether or not the same conclusion may be drawn for other disinfectants is not clear, and some bacteriologists believe that bacteriostasis involves a mechanism different altogether from the actual killing mechanism. This may be true of the type of compound which interferes with enzyme systems, but the weight of evidence in the case of the quaternaries, points to the former concept.

With the phenols, the bacteriostatic effect is far less pronounced, but it has been found, in anti-bacterial testing, that when the phenol which is carried over into the plates, is partially neutralised by the addition of ferric chloride, a lower killing power is recorded for the phenol.

In the skeleton form of testing described above, it will have been obvious that in removing a sample from the medication tube to the Petri dish, a small amount of the disinfectant solution must have been carried over with the germs, alive or dead, into the agar.

Surface-active quaternary ammonium compounds usually exhibit killing powers against most micro-organisms in dilutions of 1 part in 500 to 1 part in 10,000 or more, when the exposure time varies between 2 and 15 minutes, but where the quaternary is present only in very much smaller amounts, perhaps 1 part in 50,000 or 100,000, then the effect encountered in times of exposure up to half an hour, is usually only one of bacteriostasis.

It must be emphasised that while the bacterial cell is in a condition of bacteriostasis, other secondary changes can take place inside the cell due to interference with nutrition or respiration, so that the cell eventually dies. Therefore, it seems reasonably safe to draw the conclusion that the quaternary ammonium compounds in higher concentrations are bactericidal, and in very low concentrations exhibit bacteriostasis.

When methods of testing quaternaries are discussed, the various methods which have been employed to overcome their residual bacteriostatic effect will be described, although these are intended to relate to determining the actual killing power. In practice, the bacteriostatic function is quite an important one.

The main difficulty encountered in food processing and distribution is not that presented by a few thousand organisms per square foot of surface, but by the millions of germs which can develop from those few thousand in the course of a relatively short space of time when conditions are favourable. Therefore, if only bacteriostatic effect is present, the failure of those organisms to develop will prevent either danger to health or the spoilage of the product, according to which aspect is important.

The menace of spores is also, to a certain extent, removed by the fact that the quaternaries, although in common with other disinfecting agents being weaker in their action against spores than vegetative organisms, exert a considerable bacteriostatic effect upon them, and thus prevent their development even when they are brought again into an environment which is satisfactory for their growth.

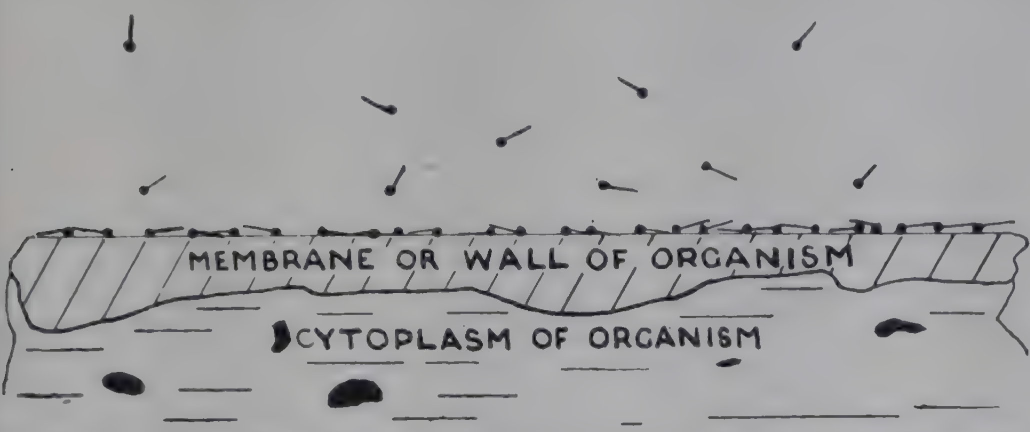
Finally, it may be of interest to attempt to visualise the action of the surface-active cation on the micro-organism. In Chapter II the physical properties of surface-active compounds were discussed, and it was seen that the boundaries of solutions containing them were packed with these molecules into a boundary layer which had certain definite properties, such as high wetting, penetrability and a low surface tension. It was also shown that with any solution, the creation of a new surface or interface with the quaternary solution results in the adsorption of the surface-active molecules to the new interface. In Fig. 13 it will be seen that this results in a layer of the surface-active quaternary ions forming on the cell; because these adhere strongly, it may be assumed that the whole of the cell wall will be completely covered by an interlaced mass of fatty chains containing for each molecule one atom of nitrogen.

It has been assumed that the quaternaries are far more powerful in their germicidal action than anionic wetting agents, because the former are more strongly adsorbed to surfaces. That the latter fact is true is beyond doubt, since the water-attracting nitrogenous part of the quaternaries is not so strongly water-attracting as the sulphate group of anionic agents. On the other hand, it would be possible to lengthen the chain in an anionic wetting agent to such a point that the ratio between the hydrophobic and hydrophilic parts of the molecule produced an adsorption effect similar in strength to that of the quaternaries. However, there is no report as yet of such a compound being found to have strong bactericidal powers.

The fact that anionic surface-active compounds are not so strongly adsorbed, seems to indicate that the neutralisation of the

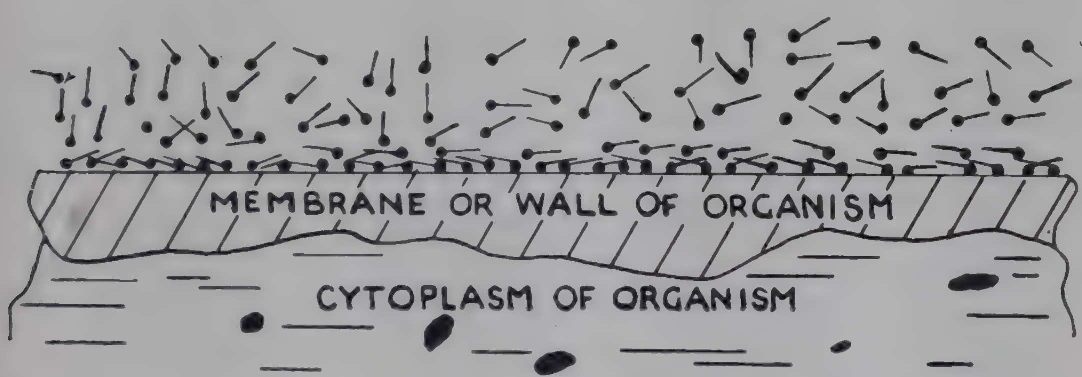
FIG. 13.

Solution of quaternary at bacteriostatic concentration (1:100,000)



(a) Portion of cell wall and cytoplasm of a micro-organism showing adsorption of surface active cations only sufficient to produce possible bacteriostasis.

Solution of quaternary at bactericidal concentration (1:5000)



(b) Heavy adsorption resulting in death of the cell.

strongly acidic sulphate or sulphonic group by a strong alkali such as caustic soda, results in a neutral salt which cannot readily react with groups in the cell wall to form un-ionized complexes, whereas the quaternary ammonium compounds which appear to behave as the salt of a strong acid with a weaker base, would have a marked tendency to form complexes with anionic groups in the cell wall, this kind of adsorption involving a chemical bond. On the other hand, it has been shown that a partial solution of the cell wall which allows the escape of certain vital constituents of the cell into the surrounding solution results in the death of the organism. So that it might be postulated, that in bacteriostasis, the physical adsorption is not sufficient to bring about the lysis of the cell wall, but that complex formation by the molecule interferes with the normal life processes and metabolism. When the concentration of the solution is brought into the bactericidal range, physical

adsorption is sufficiently heavy to bring about the rupturing of the membrane and the escape of the vital constituents, hence the cell dies fairly rapidly.

Salton, Horne and Cosslett,[43] using the electron microscope, have been able to produce some interesting proofs of this type of action. The photographs which they have published show quite clearly the effect of a solution of cetyltrimethylammonium bromide of a concentration of about one part in 10,000, on cells of *S. aureus*, *Streptococcus faecalis* and *Esch. coli*. The electron microscope shows at first the unattacked cell with its cell wall intact. After exposure to the disinfectant solution, the cytoplasm is seen to have contracted from the cell wall, this being due to the loss of certain of its constituents into the surrounding disinfectant solution, and the final stage of the operation, which can be produced more rapidly by a higher concentration of the disinfectant, shows that the cell wall has disappeared entirely. Thus, treatment of *S. aureus* suspensions with concentrations of cetyltrimethylammonium bromide of about 1 part in 1,000 for 30 minutes, strips off the cell wall almost entirely in a large percentage of the organisms. This would indicate that at first there is a definite attack on some part of the cell, disrupting the cell wall and causing the leakage of some of the vital constituents into the surrounding solution. As the action proceeds, the attack upon the cell wall becomes more widely spread, and finally this part of the organism disappears altogether, depending of course upon concentration and time of exposure to the disinfectant.

The electron micrographs reproduced in this paper are extremely illuminating, and must be considered as a valuable contribution to the study of the mode of action of quaternary ammonium compounds.

Toxicity

Having considered the mode of action of these substances against bacteria, it is not out of place to discuss their toxicity against animals and human beings. Since these substances are toxic to micro-organisms, it does not seem unreasonable to ask to what degree they are also toxic to the cells in the animal body. It is particularly important, where any substance is likely to come in contact with foodstuffs, that its toxicity should be rigorously examined.

Firstly, it may be useful to have a precise definition of what is meant by toxicity. A quotation from a recent article on toxicity by Shelanski,[3] appears to be worth while quoting for this purpose:

> In speaking of the toxicity of a substance we are merely stating what the deleterious effect of a substance is on the normal physiological response of a given biological system. A biological system may be defined briefly as any living organism of plant or animal origin. Thus the effect of a substance on the physiological response of a biological system called bacteria may vary

from the effect of the same substance on the physiological response of a biological system called man.

These effects may vary in different biological systems, or in other words, the effects on different organisms may vary. Therefore, it becomes important, once it is established that a substance will harm insects, to determine whether this same substance will harm man. Thus it is necessary to determine the toxicity of a substance. Toxicity may be defined as the sum total of all harmful effects upon an organism.

We recognise two kinds of toxicity: acute toxicity and chronic toxicity.

Acute toxicity is the manifestation of damage to the system immediately after the poison or the material has been taken into the system, either by the mouth or by the bloodstream through injection. An example of this would be the swallowing of potassium cyanide where violent toxic reactions would be evident, the victim dying in a few minutes.

Chronic toxicity, on the other hand, would be the manifestation of injury or damage to the system causing illness of some kind, which becomes only apparent gradually after the poison has been taken in very small quantities over perhaps quite a long period.

It can be mentioned that a large number of substances which are regularly taken with food or drink quite safely over long periods would probably have fatal results quite quickly if taken in large doses; for example, if an individual ate 1 lb. of ordinary common table salt straight off, the results would prove fatal, but taken in the normal way there are absolutely no toxic effects whatsoever.

Another point to be taken into consideration is whether the substance under examination is likely to enter the system by mouth, or to be inhaled as a gas, or whether it is to be introduced directly into the bloodstream by means of intravenous injection. Preparations of vaccines and chemotherapeutic agents which are used in the latter way, have to be rigorously controlled in order to prevent the accidental inclusion of substances which, once introduced into the bloodstream, would produce severe toxic symptoms or possibly death.

The quaternary ammonium compounds are not intended for use as chemotherapeutic agents, and are never likely to be introduced intravenously into the bloodstream, although mould growth in certain liquid preparations has been prevented by the inclusion of minute quantities of quaternary ammonium compounds, and these preparations have been used successfully for intravenous injection.

It is further unlikely that anybody, or any person, would take by mouth a large quantity of a quaternary ammonium compound, except under the same conditions which, on very rare occasions, have led individuals to drink disinfectant by mistaking it for a bottle of medicine, and even this condition is most unlikely to arise.

Therefore, the main interest in the toxicity of the quaternaries

is centred upon the question: "Will small traces of these compounds which may be left behind, after sterilising food equipment, adversely affect the health of people consuming foods taken from this equipment?"

In the first place, the quaternaries are rarely used for practical disinfection in concentrations higher than 1 part in 1,000, and even if no rinsing away of the disinfecting solution afterwards is undertaken, the quantity which remains on the surface would be extremely small. The food or beverage with which the vessel or equipment comes in contact will be a very considerable volume, and by comparison the amount of quaternary which could possibly get into them would be, at the highest, 1 part in several millions, and possibly in several hundred millions.

The strong powers of adsorption of quaternary ammonium compounds have already been discussed, and it will be seen that where solid foods are concerned, these minute traces would be adsorbed and completely inactivated. Where beverages and liquids are concerned, the presence of minute traces of this character can hardly have any significance, and doubtless there are other many more toxic things in other common beverages present in even greater quantities. From the practical standpoint, therefore, it cannot be anticipated that this can be a source of trouble, and yet the point is often raised by people who are contemplating their use.

Taking a further case, again an unlikely one, that some individual will drink a solution of actual disinfection strength, say 1 part in 1,000 or 1 part in 5,000, the quaternary, on reaching the stomach, would probably be adsorbed on other organic material present, and therefore could not be expected to have much effect on the system.

Shelanski's work on the toxicity of certain quaternary ammonium compounds as far as oral toxicity is concerned, lends some point to this particular phase of the discussion, and details of his experiments where dogs were, for several months, drinking solutions of various quaternaries at the level of 1 part in 5,000, the ordinary disinfection level, are worth mentioning.[4]

Four representative compounds were tested against white rats, guinea-pigs and dogs. It was found that when concentrated solutions of these substances were administered by being introduced directly into the stomachs of the test animals, toxic effects were obtained showing an L.D. 50 of about 500 milligrammes per kilogramme. The strength of the solutions thus administered were 10% and 20%, and it was found that when the animals were autopsied the concentrated solutions had had an irritating effect on the gastro-intestinal mucosa. However, when solutions of 0·1% strength were administered, white rats and guinea-pigs survived administrations of as much as 75 c.cs. per kilogramme of body weight level of administration.

These results indicate that a human being would have to take fairly large quantities of concentrated quaternary ammonium

solutions in order to suffer any toxic reactions, but that is not the angle from which the toxicity of such compounds is usually regarded. These compounds are used in dilutions which vary as outside limits from 1 in 2,000 to 1 in 10,000, and the main interest of the public health department is in knowing whether prolonged exposure or prolonged ingestion of solutions at these concentrations, will have any chronic toxic effects.

It is particularly interesting to find in this particular paper that five groups of ten dogs were used in the chronic toxicity study. Four of the groups were made to drink a 1 in 5,000 dilution of the quaternary ammonium compound as their drinking water, for a period of six months, while the fifth group of dogs was used as a control. The dogs which had drunk only the quaternary ammonium solution, were found at the end of the period to be in perfect condition from every biological aspect, and no difference was observed between the treated dogs and the dogs which had drunk merely water. The weight curves for both the experimental and the control animals were normal. After the six months, six animals from all the groups were subject to autopsy, and tissues were removed for histo-pathological study from the stomach, intestines, spleen, liver, kidney, heart, lung, pancreas and adrenal. There were no deviations from the normal controls observed in any of these tissues.

This is a most important experiment, and could hardly have been more severe or prolonged as a means of attempting to determine whether the four compounds studied had any chronically toxic effects.

The conclusions drawn by this author are, that the four quaternary ammonium compounds studied should be considered as irritants only in high concentrations. In the lower concentrations tested, the substances are apparently non-toxic to warm-blooded animals.

With regard to the point of acute toxicity which is hardly ever likely to arise, it seems clear that in high concentrations, say 5% or 10% solutions or upwards, the surface-active property of these compounds comes into play, and because of this, irritation of the mucous membranes takes place, and probably would take place on a severe scale, and similar results might well indeed be expected from any surface-active compound if taken internally in quantity.

It has been mentioned in various places that quaternary ammonium disinfectant solutions can bring about lysis of blood cells; so can soaps and ordinary anionic wetting agents, and it is a little difficult to see the significance of this particular observation under practical conditions. Admittedly, if sufficient damage is done to the mucous membrane by taking doses of concentrated quaternaries, then internal haemorrhage would be brought about, and presumably the quaternary ammonium solution would then proceed to destroy the blood cells. Once again, it must be pointed out that it is difficult to see under what conditions high concentrations of quaternaries

are ever going to be introduced into the stomach, since the only people likely to take them accidentally or intentionally would probably be mental cases or would-be suicides.

Toxicity is usually measured at three levels. Firstly, the amount necessary to bring about the death of 100% of the experimental animals being used, and this is called L.D. 100. Next, that amount of the compound which will cause the death of 50% of the animals, and this is the L.D. 50, and finally the quantity which does not produce death amongst any of the animals, which is the L.D. 0. The L.D. 50 is usually taken as the toxic level; any quantity greater than this must be regarded as likely to produce death, whereas beneath this level, greater percentage of survivals may be anticipated.

Several other workers have conducted toxicity tests rather similar to those described by Shelanski. For example, Walter,[27] who working with guinea-pigs obtained results very similar to those of Shelanski, and from the literature generally, the following general conclusion may be drawn:

As far as acute toxicity is concerned, it would seem that most quaternaries which have been studied appear to have very much the same L.D. 50 figures (about 300 mg. per kg. of bodyweight) against experimental animals such as rats or guinea-pigs. It was interesting to find that di-*n*-octyldimethylammonium bromide was also of this order of activity, although its properties differ rather considerably from long chain quaternaries, and even from its close relation, di-*n*-decyldimethylammonium bromide, which came into the same range. An American quaternary known as "Tetrosan", which contains chlorine attached to a benzyl group (alkyl dimethyl 3, 4 dichlorobenzylammonium chloride), falls into the same class, and these results when translated into terms of quantities which could be taken by a man weighing 11 stones, mean that as much as one ounce of pure quaternary could be taken at one time without any untoward reaction. The possibility of anyone doing this is about the same as the possibility of anyone taking a teaspoonful of caustic soda internally, with what results can be best left to the imagination; caustic soda and detergents based on it being frequently used in the cleaning of much food plant.

The question of toxicity should include some observations about possible irritant action on the skin. Here again Shelanski in the same paper gives some interesting data.

Skin has two main layers, one called the epidermis which is the outer layer of skin, and the dermis which is the underlying structure. The epidermis is composed of a layer of living cells with an outermost or surface layer of dead cells, which are flat in appearance and contain a fatty or waxy material called "sebum" which makes the skin surface both pliable and waterproof. This outermost layer is always being removed by friction and washing, and of course is continually replaced by the cells in the lowest layer of the epidermis,

and as these cells approach the surface, they change from living cells into the dead cells which are seen at the top.

The dermis consists of dense fibrous tissue which contains blood vessels, sebaceous glands, hair follicles and sweat glands, while the nerve endings which enable us to feel things, are also located here. The surface of this dermis is wavy with conical elevations which fit into the epidermis like fingers into a glove, so it is difficult to say that the two layers begin and end sharply, and it is better to regard them as merging one into the other.

Where the epidermis is normal and healthy and unbroken, it is effective in providing protection against irritation and bacteria. The dermis discharges a special duty, in that it regulates body temperature and performs other functions. The skin is often the first part or organ of the body to react to irritation and infection from internal sources, when a rash may develop before other symptoms become apparent.

The condition known as "dermatitis" covers a large number of skin troubles, and the term has been used very carelessly in the past to cover almost any observable appearance or condition of the skin, without due respect to any other factors, but now doctors and particularly specialists, prefer to be much more specific in their diagnosis of any apparent skin condition, rather than to rely upon the general term "dermatitis". Naturally for the issuing of a medical certificate the term "dermatitis" is still in common use, but this is a mere general classification for record purposes and insurance reasons, but further investigation at a hospital or by specialists usually results in a more precise definition.

Practically speaking, almost anything can become a source of dermatitis, *if the condition of the patient is susceptible to it*, and this makes a precise definition of industrial or occupational dermatitis very difficult. The condition of general health of the individual is a very important factor, and types of skin troubles are known where the real cause is nerves, anxiety and abnormal mental conditions.

Shelanski found that quaternary ammonium compounds may be considered as strong primary skin irritants *in concentrated solutions*. In dilutions of 0·1% or less, none of these substances may be considered as primary irritants. It was also found that they do not appear to be sensitisers, that is to say, that they do not make the skin more sensitive to other forms of irritation, whilst Schwartz[28] mentions that quaternary ammonium compounds have a use as cleaners for removing cutting oils from the skin and for the prevention of skin infections, so that there would appear to be an argument in favour of their controlled use on the skin in certain circumstances.

The author has in this section resorted in some measure to generalisations, because precise data for all the quaternary ammonium compounds are not yet available. It is, therefore, necessary to keep the question of toxicity in its true perspective, and to bear

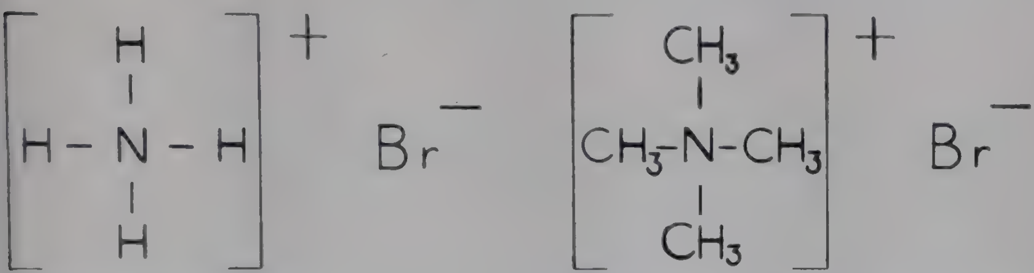
in mind that each quaternary ammonium compound should be examined independently, as it is possible that some new type of quaternary, containing some other groups, may be more toxic than these which have already been reported upon.

To summarise, it would appear reasonably safe to state that no proof has yet been found that the minute quantities likely to be ingested as a result of the disinfection of food utensils and equipment, can have the slightest harmful effect on the human organism. When the fundamental property of adsorption on to all kinds of organic matter is taken into consideration, and the extremely minute traces which are likely to remain after treatment, it seems reasonable to state, that the quaternary ammonium disinfectants are among the safest kinds of anti-bacterial agents that could possibly be applied in the food and allied industries.

CHEMISTRY

CHEMICALLY, quaternary ammonium compounds may be regarded as being derived from the ammonium salts by the substitution of organic groups for the four hydrogen atoms in the ammonium ion. (Fig. 14.)

FIG. 14.

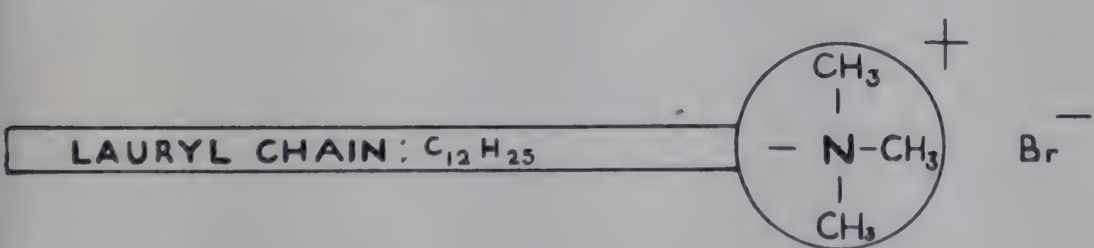


Ammonium Bromide. Tetramethylammonium Bromide – a simply non-surface active quaternary ammonium salt.

Tetramethylammonium salts can be regarded as the very simplest quaternary ammonium compounds which it is possible to make The salts may be chlorides, bromides, iodides or sulphates, etc., and the same rule will apply to the surface-active quaternary ammonium compounds which are under discussion here.

Tetramethylammonium bromide is a very soluble substance, and is not surface active. It has no use in disinfection, although in point of fact it has some physiological activity in other directions. When one of the methyl groups in a tetramethylammonium salt is replaced by a long fatty chain, such as the lauryl chain, which consists of 12 carbon atoms linked to one another with hydrogen atoms attached to each carbon atom, we have a surface-active quaternary ammonium compound with bactericidal powers. (Fig. 15.)

FIG. 15.



Lauryl trimethyl ammonium bromide a surface active germicidal quaternary ammonium compound.

Another well-known quaternary ammonium germicide is cetyltrimethylammonium bromide, and this has 16 carbon atoms in the chain and is probably one of the best known and most used quaternary ammonium compounds.

Both these substances can be regarded as derivatives of trimethylamine, a common elementary tertiary base, and if trimethylamine is treated with either lauryl or cetyl halides under certain conditions of heat and pressure, then the resulting product will be lauryl or cetyl trimethylammonium halide; in fact any tertiary amine when treated with an organic halide, will yield a quaternary ammonium salt, and the whole derivation from ammonia may be given in the following stages. (Fig. 16.)

FIG. 16.

Ammonia heated with methyl bromide under pressure to give trimethylamine:

$$NH_3 \xrightarrow{CH_3Br} NH_2 \cdot CH_3 \xrightarrow{CH_3Br} NH \cdot (CH_3)_2 \xrightarrow{CH_3Br} N(CH_3)_3$$

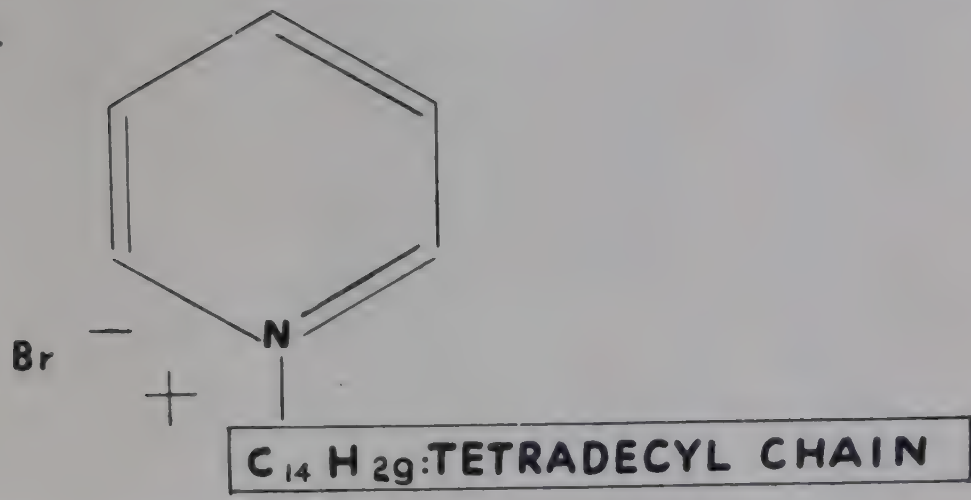
trimethylamine heated with cetyl iodide to give cetyltrimethylammonium iodide:

$$N(CH_3)_3 \xrightarrow{C_{16}H_{33}I \cdot} C_{16}H_{33}\;\begin{matrix} CH_3 \\ | \\ N-CH_3\,I \\ | \\ CH_3 \end{matrix}$$

Pyridine is a well-known tertiary base obtained from coal tar and other sources, and is relatively cheap and freely accessible. When this is treated with a long chain organic halide, a quaternary ammonium compound results. This particular kind of quaternary ammonium compound is called "pyridinium salt", and an example of a commercially produced quaternary of this type is shown in Fig. 17.

Since any tertiary amine or tertiary organic base will yield a quaternary ammonium compound, it follows that the possible number is almost infinite, and since the publication by Domagk[5] in 1935 of his discovery of the germicidal powers of a long chain alkyldimethylbenzylammonium chloride, many hundreds of more or less surface-active quaternary ammonium compounds have been produced, using not only pyridine but quinoline, *iso*quinoline, morpholine, tetrazole and many other compounds containing

FIG. 17.



Tetradecylpyridinium Bromide

nitrogen. These have been studied and reported upon in the literature. Few, if any, of these have been found to be superior to the simpler types of surface-active quaternaries, and since they would be much more expensive to produce, they cannot be regarded as having much commercial importance.

In Great Britain, the types of quaternary ammonium compounds at present being marketed, are those which either may be regarded as being directly derived from a simple aliphatic tertiary amine, or else from pyridine. In the United States, the actual number of compounds on the market is rather greater than in Great Britain, and variation in the kind of fatty chain is more commonly made.

The materials from which fatty chains are usually obtained are various, but a considerable amount is obtained from the hydrogenation of coconut oil which provides a range of alcohols. These fatty alcohols vary in chain lengths from six carbon atoms up to 18, and where the chain in a quaternary ammonium compound is merely described by the general term "alkyl", it often means that a mixture of chain lengths is being employed, as for example the product alkyldimethylbenzylammonium chloride, which is extensively marketed in the United States.

There may be variations from time to time in the amount of each particular chain length present, although perhaps not to more than 2% or 3%. Nevertheless, such variations can render more difficult chemical estimations which may have to be carried out, and may also make for variations in other properties including antibacterial activity, although it is only fair to state that the variations are not usually so great as to make a considerable difference. In the same way, other compounds which are specified as being lauryl, myristyl, cetyl, etc., may contain varying small quantities of higher or lower chain lengths, so that the commercial quaternary ammonium compounds are rarely absolutely pure from the point of view of having a definite and fixed composition.

TABLE II

COMMERCIALLY PRODUCED SURFACE-ACTIVE QUATERNARY AMMONIUM GERMICIDES

PRODUCED IN GREAT BRITAIN—(List may not be complete)

Chemical name of quaternary ammonium germicide	*Manufacturer*	*Trade name (where known)*
Di-*n*-decyldimethylammonium bromide ..	The British Hydrological Corporation ..	Deciquam
Di-*n*-octyldimethylammonium bromide ..	The British Hydrological Corporation ..	Diometam
Cetyltrimethylammonium bromide	Imperial Chemical Industries Ltd. ..	Various preparations sold as: Cetavlon, Cirrosol O.D., Vantoc A., Lissolamine A.
Tetradecylpyridinium bromide	Imperial Chemical Industries Ltd. ..	Fixanol V.R. Vantoc B.
Cetyl pyridinium bromide	Imperial Chemical Industries Ltd. ..	Fixanol C.
Lauryl pyridinium chloride .. .. .. Cetyl pyridinium chloride .. .. .. Lauryldimethylbenzylammonium chloride .. Cetyldimethylbenzylammonium chloride .. Mixed alkyldimethylbenzylammonium chloride Stearyldimethylbenzylammonium chloride ..	Leda Chemical Co. Ltd. Leda Chemical Co. Ltd. Leda Chemical Co. Ltd. Leda Chemical Co. Ltd. Leda Chemical Co. Ltd. Leda Chemical Co. Ltd.	

Continuation of Table II]

EXAMPLES OF MAIN TYPES PRODUCED IN THE U.S.A.

Chemical name of quaternary ammonium germicide	*Trade name (where known)*
Alkyldimethylbenzylammonium chlorides	Onyx B.T.C. Roccal Roclina Zephiran Zephirol
Alkyldimethyl3:4dichlorobenzylammonium chlorides	Tetrosan
Di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride	Amerse Hyamine 1622 Phemerol
Cetyltrimethylammonium bromide	CETAB
Oleyldimethylethylammonium bromide	Amerse Onyxide Quartol
Lauryldimethylchlorethoxyethylammonium chloride	Isothan OX.
Cetyl pyridinium chloride	Ceepryn
N-(higher acyl esters of colaminoformylmethyl)-pyridinium chloride	Emulsept
Lauryl *iso*quinolinium bromide	Isothan Q.15

It might be useful at this point to note the chief quaternary ammonium compounds available in this country and in the United States.

In Great Britain, lauryl and cetyltrimethylammonium compounds are in use and also lauryl, tetradecyl and cetyl pyridinium compounds. The more recently developed twin chain compounds, di-*n*-decyldimethylammonium bromide and di-*n*-octyldimethylammonium bromide, are produced in Great Britain only.

In the United States, the long chain alkyldimethylbenzylammonium compounds such as B.T.C., Roccal, Zephirol and Triton K-12, are well known, and also the alkylphenoxyethoxyethyldimethylbenzylammonium halides such as Phemerol and Hyamine 10X, as well as the long chain trimethylammonium halides as in this country.

Table II gives the chemical names and trade names, where known, of all these compounds.

It must be borne in mind that the hydrophobic carbon chain can be interrupted in various ways, for example, by means of ether or amide linkages both of which are known and used, and that the secondary groups attached to the nitrogen atom can also be altered. These secondary groups, however, do not play a very important part as far as the germicidal activity of the compound is concerned, but they do have some slight influence. It is, however, true to say that the significant group in these quaternary ammonium compounds, is that which confers the surface-active property upon the molecule, which brings this discussion to a rather fundamental point.

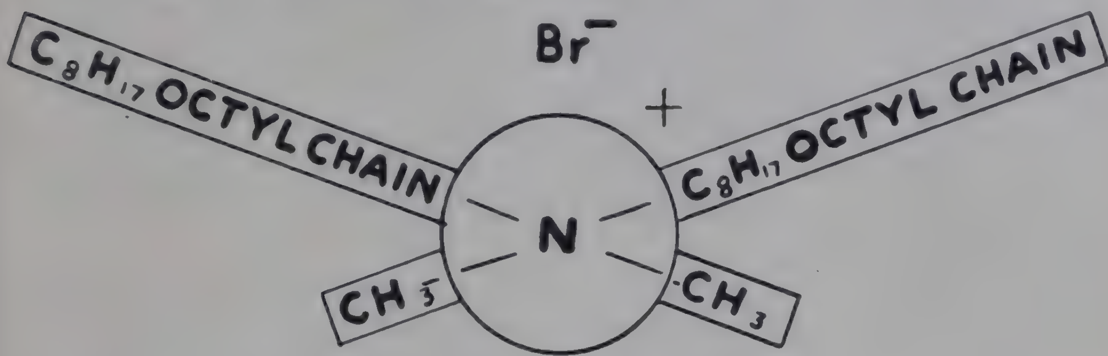
What constitutes a surface-active chain? When can it be said that the length of the carbon chain is such that it will confer surface-active properties, and some degree of germicidal activity upon the molecule?

It is not possible to make a hard and fast rule about this, because it will depend upon the water-attracting powers of the nitrogen-containing group to which the chain is attached, and this will be found to vary between simple trimethylamine residues, benzyldimethylamine residues, pyridine groupings and others. Generally speaking, the more powerful the water-attracting powers of the nitrogen grouping, the longer will the chain have to be before surface-active properties are significant. To illustrate this, let us turn to the simple alcohols.

Ordinary ethyl alcohol (C_2H_5OH) contains a very short organic group, the ethyl group, attached to the hydroxyl group which is water-attracting. In this molecule the water-attracting powers of the hydroxyl group unquestionably predominate, and it cannot be said that surface-active properties are noticeable. If the chain is lengthened through propyl (C_3H_7) and butyl (C_4H_9) to amyl (C_5H_{10}), it is found that the water-attracting powers of the hydroxyl group in amyl alcohol are being overshadowed by the organic amyl group which is now big enough to make an effort to pull the molecule

out of solution, and therefore surface-active properties can be found in amyl alcohol, i.e. it is adsorbed to surfaces and can be made to reduce the surface tension of water when present in relatively small quantities.

If this experiment is repeated, using instead of the alcohols the primary amines, ethylamine, propylamine, butylamine and amylamine, it will be found that even with normal amylamine there is much less evidence of surface activity than with normal amyl alcohol, and this will be a reflection of the fact that the primary amino group in these compounds is much more powerfully water attracting than the alcoholic hydroxyl. Therefore, to obtain surface



(a) Di-n-Octyldimethylammonium bromide.

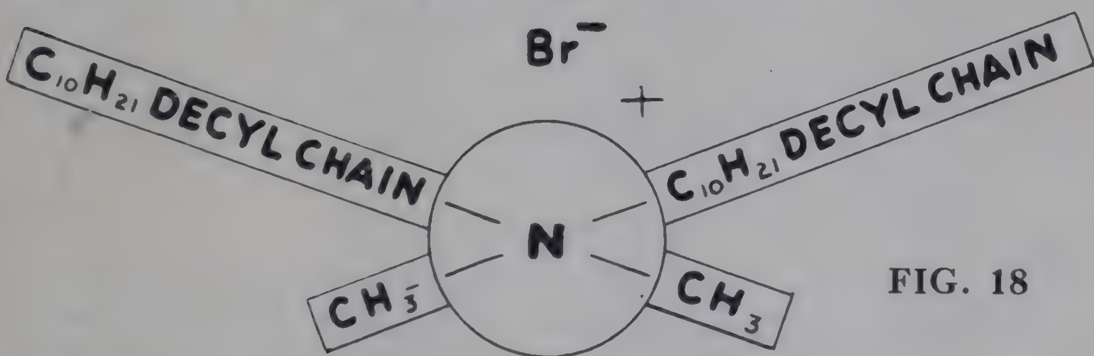


FIG. 18

(b) Di-n-decyldimethylammonium bromide.

activity in the latter series, the chain must be lengthened still further. It is important to bear in mind, therefore, that the nature of the nitrogen grouping can play a significant part, and while it would be reasonable to compare various long chain trimethylammonium compounds with each other for surface activity, it would be wrong to assume that lauryl pyridinium chloride manifests the same degree of surface activity as does lauryl trimethylammonium bromide.

Germicidal quaternary ammonium compounds can be classified according to:

(*a*) The number of chains in the molecule which contribute towards surface activity.

(*b*) Whether these chains contain any unsaturated linkages such as in the oleyl group.

(*c*) Whether such surface-active chains contain other elements such as chlorine atoms, or other groups such as amide, ester or ether linkages.

(*d*) Whether the non-surface-active groups contain elements other than carbon and hydrogen.

It must be borne in mind that these compounds can contain more than one chain contributing towards surface activity, for example in the twin chain compounds, di-*n*-octyldimethylammonium bromide and di-*n*-decyldimethylammonium bromide (Fig. 18), there are two chains which contribute towards surface activity, the difference between the single chain type of compound and the twin chain, being that with the twin chain, a greater total number of carbon atoms is possible in the surface-active chains than in the single chain compounds, without bringing the compound to such a low degree of solubility that it has no practical value or becomes ineffective as a bactericide.

Compounds which are derived from pyridine would form another group, also those derived from any other kind of tertiary amine.

One of the most confusing things about the mass of published data in this field, particularly in that section dealing with the application and uses of these compounds, has been the tendency for generalisation, statements being made that quaternary ammonium compounds have this or that property, while in fact the particular property referred to may only have been noticed with one compound, and it cannot be too strongly emphasised that such generalisations are misleading.

Reactions

The stability of quaternary ammonium compounds towards heat, alkalis and other agents varies between the classes mentioned above, for example, warming alkyltrimethylammonium bromide with a dilute caustic soda solution, does not appear to cause any observable breakdown, but a pyridinium salt under the same conditions in solution, turns a deep brownish red, and a strong odour of liberated pyridine is soon noticed.

FIG. 19.

$$C_{16}H_{33}N(CH_3)_3Br \xrightarrow{KOH} C_{16}H_{33}N(CH_3)_3OH$$

Cetyltrimethylammonium bromide — Cetyltrimethylammonium hydroxide

The surface-active quaternary ammonium salts can be converted into quaternary ammonium bases or hydroxides. (Fig. 19.)

The most convenient way of doing this is by dissolving the salt in

alcohol, and adding the calculated quantity of sodium or potassium hydroxide also dissolved in alcohol, and then filtering off the precipitated potassium or sodium halide. On distilling off the alcohol, the quaternary hydroxide will remain. This will be found to have properties very similar to that of the salt, and little variation in anti-bacterial activity will be noticed. The hydroxides, however, are rather more easily decomposed than the salts, although again the conditions under which it is necessary to work to bring about decomposition, are not met with in practical usage. The salts themselves can be degraded quite easily in the laboratory to give a tertiary amine, thus cetyltrimethylammonium bromide, heated in a vacuum distillation apparatus where the pressure is reduced to about one millimetre, will melt, and an amine will distil over which proves to be the tertiary amine cetyldimethylamine, a molecule of methyl bromide being eliminated in the process.

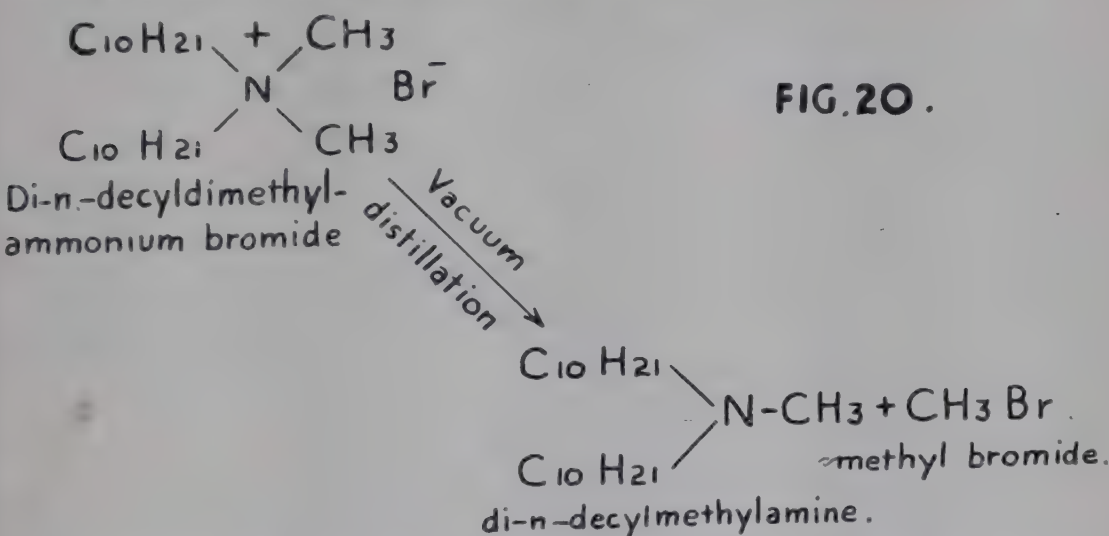


FIG. 20.

Similarly the related hydroxide will decompose giving methyl alcohol. Di-*n*-decyldimethylammonium bromide, under the same conditions, gives di-*n*-decylmethylamine. (Fig. 20.)

Since these substances ionize in solution, they will react with many anionic materials of both an organic and inorganic nature, the principle being that of double decomposition, and the complex formed between anion and cation is often precipitated. In some cases, however, no apparent reaction can be noticed, but keeping the solutions for a few days often results in a precipitate being formed.

It must be borne in mind that solutions of these compounds have properties common to many colloidal electrolytes, and this often results in delay in the appearance of the precipitate.

Once again it is important to define the chemical nature of the quaternary ammonium compound, as considerable variations in behaviour with respect to anionic substances between different compounds can be noted.

In the author's laboratory, experiments have been carried out using, firstly, the long chain cetyl or *n*-hexadecyltrimethylam-

monium bromide, and two twin chain compounds, di-*n*-decyldimethylammonium bromide and di-*n*-octyldimethylammonium bromide. The conditions of the experiments were as follows:

Five c.cs of each of the three quaternary ammonium compounds in 0·2% solution were mixed with 5 c.cs. of sodium metasilicate solution, 5 c.cs. of trisodium phosphate, 5 c.cs. of sodium hexametaphosphate, 5 c.cs. of sodium oleate, 5 c.cs. of sodium lauryl sulphate (all at 0·2%), and the results are given in Table III. In this table and in Table IV, (page 54)the following method is used to describe the results of the bacteriological tests applied to the mixtures. Where no growth was found on the plate after incubation, the sign — is used, slight growth +, moderate growth ++, heavy growth +++, and very heavy growth ++++. For the sake of brevity, the three quaternary ammonium compounds are designated as follows:

di-*n*-octyldimethylammonium bromide = A
di-*n*-decyldimethylammonium bromide = B
n-hexadecyltrimethylammonium bromide = C

TABLE III

Quaternary salt	*Reagent*	*Appearance after* 48 *hours*	*Bacteriological test of* 1 *c.c. of the mixtures (well shaken) against* Esch. coli. *Exposure time* 10 *minutes*
A	Sodium metasilicate	Clear	—
B	Sodium metasilicate	Slight crystalline precipitate . .	+
C	Sodium metasilicate	Heavy flocculent precipitate . .	+
A	Trisodium phosphate	Clear	—
B	Trisodium phosphate	Clear	—
C	Trisodium phosphate	Slight precipitate	—
A	Sodium hexametaphosphate . .	Opacity	—
B	Sodium hexametaphosphate . .	Oily suspension . .	—
C	Sodium hexametaphosphate . .	Moderately heavy precipitate . .	—
A	Sodium oleate . .	Oily suspension . .	+ +
B	Sodium oleate . .	Oily suspension . .	+ + +
C	Sodium oleate . .	Slight opacity . .	+
A	Sodium lauryl sulphate	Oily suspension . .	+
B	Sodium lauryl sulphate	Slight precipitate	+ + +
C	Sodium lauryl sulphate	Slight opacity . .	+ +

This means in effect that quaternary ammonium compound solutions at the rate of 1 part in 500 of water were mixed with anionic materials in the same concentration and allowed to stand for 48 hours. Against the three inorganic substances, di-*n*-octyldimethylammonium bromide is quite unaffected. Di-*n*-decyldimethylammonium bromide is slightly precipitated, but the long chain *n*-hexadecyl(cetyl)trimethylammonium bromide is affected very considerably. Nevertheless, the bacteriological results show only slight inactivation of di-*n*-decyldimethylammonium bromide and *n*-hexadecyltrimethylammonium bromide with sodium metasilicate. With sodium oleate, however, visual precipitation or suspension seems to take place with the two twin chain compounds rather more than with the long chain compound, and bacteriological inactivation seems to have proceeded much further, but even so it is by no means complete.

In Table IV, 0·2% solutions of the quaternary ammonium compounds are used, 4·5 c.cs. being taken on each occasion and 0·5 c.c. of 5% Na_2CO_3 being added. The same anionic substances were used again, 4.5 c.cs. of 0·2% solutions being added to the quaternary plus sodium carbonate mixture.

In this experiment, di-*n*-octyldimethylammonium bromide is consistently the best throughout with little to choose except in the last case, between the *n*-hexadecyl compound and the di-*n*-decyl. It should be noted that the long chain *n*-hexadecyl compound and di-*n*-octyldimethylammonium bromide both have the same total of carbon atoms in their surface-active chains, i.e. 16, yet their behaviour is strikingly different with respect to the compounds against which they were tested. This difference is, without doubt, due to the greater solubility of the di-*n*-octyl compound tending to make a more soluble complex with the inorganic salts, but with the organic anionic compounds, the complex is less soluble. The long chain *n*-hexadecyl compound visually appears to be much more incompatible with inorganic substances of this type, but on the other hand, shows improved behaviour with the organic materials.

These experiments serve to show how very important it is to deal with each quaternary ammonium compound separately. The pyridinium salts, for example, also show a tendency to make more soluble complexes, and further, although a heavy precipitate of complex may be formed, it does not mean that this mixture is necessarily inactive as far as antibacterial functions are concerned.

The question of the reaction of quaternary ammonium compounds with anionic substances is one of great practical importance, since when they are used for sterilising, the presence of an oppositely charged ion can, in theory at any rate, result in the loss of germicidal potency, and if, for example, a wetting agent or a soap solution has been used for cleaning a surface before its disinfection with a quaternary ammonium solution and without any previous rinsing, then results may be very poor, even dangerously so.

TABLE IV

Quaternary salt	*Reagent*	*Appearance after 48 hours*	*Bacteriological test of 1 c.c. of the mixtures (well shaken) against* Esch. coli. *Exposure time 10 minutes*
A	Sodium metasilicate	Clear	+
B	Sodium metasilicate	Slight fine flocculent precipitate	—
C	Sodium metasilicate	Light fine flocculent precipitate	—
A	Trisodium phosphate	Clear	—
B	Trisodium phosphate	Clear	—
C	Trisodium phosphate	Light fine flocculent precipitate	—
A	Sodium hexametaphosphate . .	Clear	—
B	Sodium hexametaphosphate . .	Slight cloudiness	+
C	Sodium hexametaphosphate . .	Heavy solid precipitate . .	+
A	Sodium oleate . .	Light cloudiness	—
B	Sodium oleate . .	Very slight precipitate . .	+ +
C	Sodium oleate . .	Cloudiness . .	+ +
A	Sodium lauryl sulphate	Cloudiness with oily droplets . .	—
B	Sodium lauryl sulphate	Faint mistiness . .	+ + +
C	Sodium lauryl sulphate	Heavy cloudiness	—

A further reason for the importance of this aspect is that it may be required to compound a quaternary ammonium compound with inorganic detergent salts in order to make a bactericidal detergent, or at any rate, a detergent which will have positive germicidal qualities independent of those which may be due to the alkaline salts themselves.

As previously mentioned, boiling with caustic alkali will tend to decompose any quaternary ammonium compound dependent upon the length of time the boiling takes place and the strength of the solution, but apart from this, it is quite possible to have caustic soda present in a detergent mixture and a quaternary ammonium compound at the same time, but for preference pyridinium salts should not be used for this purpose. Sodium carbonate would appear to be perfectly safe for this function, but if it is proposed to use either silicates or polyphosphates, then careful experiments should be carried out to determine what proportion of the salt, if

any, may safely be used with the quaternary ammonium compound in question to prevent inactivation or precipitation.

While, as results in the above tables tend to show, visual precipitation does not necessarily mean complete anti-bacterial inactivation, it does mean that the possibility of complete inactivation is present, particularly in view of the fact that practical conditions may result in other substances being dissolved in the solution, which will tend to upset the balance more completely. The same observations apply to organic anionic materials; whereas the sodium salts of many organic acids will be found to be entirely compatible with nearly all quaternaries, the tendency is for the larger molecules not to be so, and here again it is a question of the individual compound and the exact quaternary against which it is being tested. It does not always mean when two such test solutions are mixed together that, because there is no sign of any mistiness or precipitate, the solution has not been inactivated; but usually visual clarity means that the substances are bacteriologically compatible.

Quaternary ammonium compounds often present problems in solubility. Reference to the formation of ionic micelles has already been made, and other complexities due to the physical properties of these molecules can also be envisaged. The tolerance of some solutions of quaternaries to the addition of a common ion is remarkably low, and whereas N,N,-dioctyl-N-methyl-ethyl acetoammonium chloride (unpublished data) easily gives a 10% or 15% solution in distilled water, the addition of a few drops of dilute HCl is sufficient to cause the throwing out in syrupy form of a great deal of the quaternary. This compound is so sensitive that dilute solutions of 0·5%, which are perfectly clear with distilled water, show opacities with hard waters which vary directly according to the total solids content of the water. It might be possible, with the use of this compound, to devise a turbidimetric test for hard waters, although the turbidity would not be related so much to the total hardness, as to the total dissolved solids.

When considering the question of solubility of these substances, it must be admitted that even some apparently true solutions are in fact colloidal dispersions, and while this enables the product to be used and generally treated in every way as a solution, it may be misleading to say that the substance is really in true solution in the same way that sugar or salt can be. True solubility will occur at some definite point of concentration, and this will depend upon the size and configuration of the molecule. In this chapter, reference will be made to one quaternary being more soluble than another, and where this occurs it is intended to refer to apparent solubility, whether it is a true solution or a colloidal dispersion.

Effect of Hard Waters

The effect of hard waters on the germicidal activity of some quaternaries is probably caused through similar effects on a smaller

scale, and where these substances are being used in a fairly high dilution, inactivation by very hard waters, to some degree, can be explained fairly readily on this basis. In the American literature, several references have been made to this difficulty, notably Ridenour and Armbruster,[6] Johns,[7] and Mueller and Seeley.[8] All these workers were using quaternary ammonium compounds containing only one single long chain. Generally, the picture given by these workers is quite reliable, and it seems obvious, that in the harder waters some loss of germicidal action must be anticipated. This is most particularly applicable to the Gram-negative rather than to the Gram-positive type of organism; for example, there is a difference in susceptibility to interference in hard waters between the Gram-positive *S. aureus* and the Gram-negative *Esch. coli*. In the author's laboratory, this fact has also been confirmed, but a difference was found also between the twin chain compound, di-*n*-decyldimethylammonium bromide and the long chain *n*-hexadecyltrimethylammonium bromide.

Using both the quaternaries at a dilution of 1 in 20,000 with an exposure time of ten minutes, and using a range of waters from distilled water to 51 parts per 100,000 hardness with *S. aureus* as the experimental organism, it was found that nearly five times the number of survivors were counted for the *n*-hexadecyltrimethylammonium bromide test than for the di-*n*-decyldimethylammonium bromide after 25 parts per 100,000. With *Esch. coli* the log count of survivors was plotted against the dilution of quaternary for the same two compounds and using the same water. One anomalous result was found in which di-*n*-decyldimethylammonium bromide, at 47 parts per 100,000, showed more survivors than the same compound when used in water of 51 parts, but the difference between the twin chain compound and the long one was very marked in this experiment. Here again, the greater solubility of di-*n*-decyldimethylammonium bromide, although it has four carbon atoms more in its surface-active chains than the 16 in *n*-hexadecyltrimethylammonium bromide, probably plays an important part.

Ridenour and Armbruster studied the effect of common hard water cations against a quaternary ammonium compound of the single long chain type, and found that Mg^{++} Ca^{++} ions had considerable interfering action against the test organisms, but that the anions SO_4, NO_3 and Cl had little effect. The anions PO_4 and CO_3 actually had a beneficial effect on the anti-bacterial powers of the particular quaternaries studied. The latter two anions were probably not the real cause of this increased activity so much as the increased alkalinity of the solutions containing them. This is a fairly well recognized fact, and all kinds of surface-active compounds seem to be affected similarly.

Many anionic wetting agents which have only low anti-bacterial activity in neutral solution, have their germicidal value considerably increased as the *p*H of the solution rises between 10 and 12. This

TABLE V

n-HEXADECYLTRIMETHYLAMMONIUM BROMIDE

Solution	Dilution of Q.A.	Suspensions per c.c.		Survivor counts per c.c.	
		S. aureus	*Esch. coli*	*S. aureus*	*Esch. coli*
Magnesium sulphate 0·1% solution ..	1/12,000	30,000,000	30,000,000	600	6,000
	1/14,000	30,000,000	30,000,000	1,400	20,000
	1/16,000	30,000,000	30,000,000	200	12,000
	1/20,000	30,000,000	30,000,000	500	17,000
Cadmium sulphate 0·1% solution ..	1/12,000	30,000,000	30,000,000	900	100,000
	1/14,000	30,000,000	30,000,000	1,000	200,000
	1/16,000	30,000,000	30,000,000	2,000	150,000
	1/20,000	30,000,000	30,000,000	2,000	200,000
Calcium sulphate 0·1% solution	1/12,000	60,000,000	60,000,000	400	300,000
	1/14,000	60,000,000	60,000,000	500	300,000
	1/16,000	60,000,000	60,000,000	1,000	400,000
	1/20,000	60,000,000	60,000,000	1,000	400,000
Magnesium chloride 0·1% solution ..	1/12,000	60,000,000	60,000,000	1,900	16,000
	1/14,000	60,000,000	60,000,000	1,700	20,000
	1/16,000	60,000,000	60,000,000	2,500	160,000
	1/20,000	60,000,000	60,000,000	30,000	50,000
Cadmium chloride 0·1% solution ..	1/12,000	60,000,000	60,000,000	600	40,000
	1/14,000	60,000,000	60,000,000	3,000	42,000
	1/16,000	60,000,000	60,000,000	30,000	50,000
	1/20,000	60,000,000	60,000,000	25,000	20,000
Calcium chloride 0·1% solution	1/12,000	60,000,000	60,000,000	200	100,000
	1/14,000	60,000,000	60,000,000	400	100,000
	1/16,000	60,000,000	60,000,000	400	150,000
	1/20,000	60,000,000	60,000,000	600	160,000

may therefore be regarded as a synergism between *p*H and surface activity. It would appear that all quaternary ammonium compounds similarly show greater killing power in solutions of higher alkalinity.

In the author's laboratory, a similar investigation was carried out, using the sulphates and chlorides of magnesium, cadmium and calcium. These compounds were used to examine the possible effect of certain bi-valent metal ions in solution, and they were tested against solutions of two quaternary ammonium compounds, the twin chain compound di-*n*-decyldimethylammonium bromide being compared with the single long chain *n*-hexadecyltrimethylammonium bromide, the bi-valent salts being used at a dilution of 0·1%. The concentrations of the quaternaries tested, varied from 1 part in 12,000 to 1 part in 20,000 of water, which are quite high use

dilutions for these compounds. Again the test organisms were *S. aureus* and *Esch. coli* at a very high concentration, in some cases as much as 150,000,000 organisms per c.c. It was found that the actual percentage of surviving organisms was very small, but the results were interesting, in that the twin chain compound was found to be much less affected by the presence of these salts than was the single long chain type.

The results of the experiments are given in Tables V and VI.

TABLE VI

DI-*n*-DECYLDIMETHYLAMMONIUM BROMIDE

Solution	*Dilution of Q.A.*	*Suspensions per c.c.*		*Survivor counts per c.c.*	
		S. aureus	*Esch. coli*	*S. aureus*	*Esch. coli*
Magnesium sulphate 0·1% solution ..	1/12,000	150,000,000	120,000,000	0	1,500
	1/14,000	150,000,000	120,000,000	0	1,500
	1/16,000	150,000,000	120,000,000	0	2,000
	1/20,000	150,000,000	120,000,000	600	2,000
Cadmium sulphate 0·1% solution ..	1/12,000	160,000,000	160,000,000	0	1,500
	1/14,000	160,000,000	160,000,000	0	700
	1/16,000	160,000,000	160,000,000	700	3,000
	1/20,000	160,000,000	160,000,000	800	2,000
Calcium sulphate 0·1% solution	1/12,000	150,000,000	120,000,000	0	0
	1/14,000	150,000,000	120,000,000	0	100
	1/16,000	150,000,000	120,000,000	0	3,000
	1/20,000	150,000,000	120,000,000	400	5,000
Magnesium chloride 0·1% solution ..	1/12,000	60,000,000	80,000,000	0	0
	1/14,000	60,000,000	80,000,000	0	3,000
	1/16,000	60,000,000	80,000,000	0	700
	1/20,000	60,000,000	80,000,000	400	700
Cadmium chloride 0·1% solution ..	1/12,000	60,000,000	80,000,000	0	0
	1/14,000	60,000,000	80,000,000	0	2,900
	1/16,000	60,000,000	80,000,000	300	3,200
	1/20,000	60,000,000	80,000,000	400	3,600
Calcium chloride 0·1% solution	1/12,000	160,000,000	160,000,000	0	1,300
	1/14,000	160,000,000	160,000,000	200	2,100
	1/16,000	160,000,000	160,000,000	1,000	1,700
	1/20,000	160,000,000	160,000,000	1,700	2,100

Once again the effect against *S. aureus* was not appreciable, but when the Gram-negative *Esch. coli* was involved, survival counts were larger, and with *n*-hexadecyltrimethylammonium bromide, were often in the hundreds of thousands.

As far as could be seen from these experiments, the anion had

no effect, which is the conclusion drawn by Ridenour and Armbruster, and whereas against di-*n*-decyldimethylammonium bromide, all the three cations seem to have the same order of effect, in the case of *n*-hexadecyltrimethylammonium bromide, the magnesium ion did not interfere so much as the cadmium and calcium ions. It must be remembered that the inoculi used were phenomenally high, and that under practical conditions of sanitation, no such figures would be encountered except in very unusual circumstances.

Therefore, however much laboratory tests point to interference by these ions and hard waters, it seems doubtful whether much practical significance need be attached to this fact when relating it to ordinary working conditions.

Non–foaming Quaternary

The twin chain compound, di-*n*-octyldimethylammonium bromide, was developed for a special purpose. This was to provide a quaternary ammonium compound which could be used with mildly alkaline detergents in conditions where it was important to exclude foaming. Reference has already been made to the fact that surface-active agents of all classes in common use produce more or less stable foams, and whereas shortening the chain in the single chain type of quaternary would eventually result in a compound which did not form a stable foam, the anti-bacterial activity of such a compound would be so low as to render it useless for the purposes of disinfection. The problem, therefore, was to synthesise a molecule which, while retaining the essential antibacterial activity, would by its other properties militate against formation of a stable foam in concentrations lower than 0·3%. These conditions were found to be fulfilled by di-*n*-octyldimethylammonium bromide, and its practical use has been reported elsewhere.[9]

The two main reasons for these properties would appear to be, greater solubility with a slower rate of migration to interfaces, and the molecular configuration in which the twin chains do not lend themselves so readily to the formation of an elastic film, as do the longer chains in the single chain type molecule. Its greater resistance to precipitation and inactivation can also be ascribed to the first-mentioned property. By extending the chains by two carbon atoms each, some modification of these properties has been achieved, and power of foaming is found to return. To compensate for this, in di-*n*-decyldimethylammonium bromide there is a very much greater anti-bacterial power, although the use of the di-*n*-octyl compound is almost invariably in alkaline mixtures where the pH is greater than 9·5 or 10, thereby increasing its normal killing power by the additional alkalinity effect to which reference has already been made.

Commercial Preparations and Solubility

Solubility of quaternary ammonium compounds generally is a matter of great importance, and with the single long chain type,

insolubility is achieved when the surface-active chain approaches 20 carbon atoms or equivalent in length.

The question of water solubility enters in several ways. Firstly, there is the question of the type of product which is to be marketed commercially. Many of the single chain quaternaries are sold as powders, but some, notably the pyridinium salts, being rather more soluble, are prepared as liquid preparations. For practical use the liquid preparation is always the more satisfactory, enabling disinfecting solutions to be made up much more rapidly and certainly than where a powder has to be dissolved. In order to prepare solutions of some of the less soluble substances, the use of alcohols and acetone has been resorted to, but rather more in the American products than in those on sale in Great Britain.

Physical properties of solutions of the compounds have been utilised in more than one case to bring about a homogeneous preparation of a liquid nature, and it is sometimes found convenient to use a more soluble pyridinium compound in conjunction with other quaternaries of higher bacterial activity but lower solubility, in order to produce a satisfactory solution of medium concentration for marketing.

Some preparations of quaternaries are sold as suspensions, but here the disadvantage is, that on storage and particularly in cold weather, the heavy sludge separates out, which may under adverse conditions, result in a paste-like semi-crystalline product which has to be warmed before it can be stirred up sufficiently to be poured out. In the same way, the quaternary ammonium compounds at present marketed, vary in their solubility in organic liquids such as ethyl ether. Where the chain or chains are sufficiently long, an un-ionized quaternary salt can often be dissolved in ethyl ether, and solubility in petrol is by no means unknown. The addition of a little water will produce ionized forms and micelles, and then for all practical purposes the molecule exhibits insolubility towards this solvent. In the related field of the fatty amines also, unusual solubility of salts in petrol is occasionally found, for example, the hydrochloride of 1-heptadecylamine exhibits such a solubility.

All the commercial quaternary ammonium compounds are readily soluble in alcohol and acetone, and many of them are soluble also in benzene.

Corrosion

Solutions of quaternary ammonium compounds, when used for disinfection, are generally recognised as having low powers of corrosion. Here again the importance of avoiding general statements can be emphasised, since commercial preparations of different quaternaries often show greater or less powers of corrosion. Certainly, solutions of from 5% to 10% would appear, on the whole, to be far more corrosive than either the lower concentrations used in practice or the higher concentrations which may be on sale for the

convenience of reducing bulk. There are a number of factors which have to be taken into consideration when considering the problem of corrosion.

There is the possible presence of inorganic halides in many preparations, and the greater number of quaternaries sold themselves contain halide for the anion. There is also the surface-active nature of the preparation as a factor.

In experiments carried out with mild steel, it is easy to demonstrate that the greatest degree of corrosion takes place immediately above the solution level of the quaternary solution, in which most of the iron has been immersed. That portion of the mild steel which is below the surface, shows very little tendency to corrosion compared with that immediately above. The same was found to be largely true in the case of copper and brass, brass being relatively little affected. Generally speaking, stainless steel and aluminium would appear to be very resistant to corrosion from these solutions, and some workers have reported that water has more corrosive effect against some kinds of iron, than has a quaternary ammonium solution at a strength of 10%.[10] With copper, corrosion usually manifests itself in the typically green film of copper halide, and with solutions of di-*n*-decyldimethylammonium bromide, as used for actual disinfection, this effect takes place almost solely above the surface.

Many surface-active solutions other than cationics, will exhibit this power of facilitating some degree of corrosion above the solution line, this being due to the cleaning and wetting power of the solution removing from the surface any protective film of grease, and facilitating the solution of oxygen in the aqueous film with which it is replaced.

Against many other materials commonly met with in food processing, solutions of quaternaries have no effect, glass, plastics and fabrics—of many kinds being quite unaffected.

With regard to rubber, synthetic rubbers will often be found to become soft and sticky on the surface, but pure rubber does not suffer so readily from this disadvantage. In many food and beverage plants, rubber hoses are used quite considerably, and often the outside of such hoses is finished with a canvas cover which is finally impregnated and coated with a protective paint. The inevitable effect of immersion in a disinfectant solution of a cationic germicide is to cause this outer covering to become sticky, the reason for this being that the adsorbed film of quaternary molecules tends partially to be dissolved in, and to be dissolved by, the external layer of the paint or protective preparation.

For the purpose of inhibiting any possible action on metals, sodium nitrite and sodium carbonate have been found useful, but it may not be convenient either to incorporate these products in the commercial preparation, or to utilize them when disinfecting solutions are being made up.

Chemical Testing Methods

The application of quaternary ammonium compounds has led generally to a demand by their users for effective methods of control, the important thing being, to know when the solution being used is at its correct strength, and also in those cases where the solution is being reused to determine when it may be expected to have lost its efficiency. In addition, it is sometimes required to know the strength of a stock solution, or even to check on a commercial product in order to determine whether the concentration conforms to specification.

Those users who maintain their own chemical laboratories are in a better position to utilise methods which have been developed in the last few years, and which enable very reliable results to be obtained, even when the quaternary is in high dilutions of over 1 part in 10,000 parts of water. Estimations of a few parts per million are now possible by more than one method. On the other hand, users who do not maintain a chemical laboratory, frequently require some simple and effective field test which will enable them to know whether the solution that they are using is of the correct concentration. This request is a much more difficult one to satisfy, but even so considerable progress in field test methods has been made in recent years.

Lastly, there is the academic aspect by which certain laboratories, for example, official institutions, public health departments and hospitals may require to know, not only how much of the cationic germicide is present in a preparation, but also what its chemical constitution might be. Further, the same laboratories may require to endeavour to detect the presence of these compounds in foodstuffs, possibly the most difficult demand which can be made upon analytical chemistry in this field.

The methods so far developed and found to be useful really fall into three classes, argentimetric, sulphophthalein dye colour change and extraction methods, and lastly precipitation procedures.

In argentimetric methods, the halide anion is estimated and from it the concentration of quaternary present is deduced. The argentimetric method used in conjunction with one of the methods given below will enable the actual amount of inorganic halide present to be determined. An estimation for total halide when taken in conjunction with the results obtained by either the Hartley-Runnicles,[11] Auerbach[12] or Epton[13] methods (which give the amount of surface-active material present) will, by converting the result of the latter method into percentage halide, enable by subtraction the actual amount of inorganic halide to be determined. Various kinds of indicators to assist the argentimetric procedure have been adumbrated, and possibly the best of these are eosin, dichlorfluorescein, and sometimes potassium chromate.

The second method utilises the fact that the sulphophthalein indicators have their colour displaced to the alkaline side by surface-active cations. A related method is based on the formation of a

coloured dye complex which is extracted by a solvent, and the colour in the solvent measured by a photometer and compared with a standard solution.

In the case of the first class of estimation, the main difficulty is, that although halide is the common anion in most quaternary preparations, varying quantities of inorganic halides are also often present, so that the titration will show a higher quaternary content in the solution than is actually present. Another difficulty may be, that during the preparation of the quaternary, some percentage of the halide has been converted into the quaternary hydroxide, so that it may be anticipated that argentimetric methods will give a lower reading result than other more reliable methods since developed.

An important member of the second class of test is that devised by Hartley and Runnicles. Here the blue-coloured salt formed by bromphenol blue with surface-active cations, is titrated with an anionic substance until the colour changes back to violet or purple. The general method for this may be given here:

A solution of a purified anionic surface-active agent such as sodium lauryl sulphate is prepared, and this solution should be at the same level or concentration as the quaternary ammonium solution to be tested. Thus, if the quaternary is being used at 1 part in 2,500 of water, then the anionic agent should also be at the same dilution. To 20 c.cs. of the quaternary ammonium solution, 1 c.c. of bromphenol blue of 0·01% solution (made slightly alkaline with ammonia) is added. Titrate this with the anionic wetting agent solution until the colour changes to purple. This method is not very accurate where dilutions greater than 1 part in 3,000 or 4,000 are used, but it is one of the accepted and standard methods for this estimation.

The Auerbach method uses bromphenol blue to form a colour dye complex with a quaternary ammonium salt, and this is extracted with a non-polar organic solvent. The amount of colour produced in a carefully measured amount of solvent (ethylene dichloride and benzene are the usual liquids), is compared in a photometer with a standard solution of the coloured complex formed from a definite known amount of quaternary ammonium compound.

The Auerbach method is briefly as follows:

Adding 1 c.c. of 0·04% bromphenol blue solution and 2 c.cs. of 10% sodium carbonate solution to 2 c.cs. of a solution containing about 0·2 mg. of the quaternary salt, and diluting with about 50 c.cs. distilled water in a 125 c.cs. glass-stoppered flask. After adding exactly 10 c.cs. of ethylene dichloride, the flask is shaken gently for one minute. When the layers have separated, the aqueous layer is aspirated and the coloured ethylene dichloride layer transferred to a dry test tube to which 0·5–1·0 g. dry sodium sulphate is added and mixed. The clarified coloured solution is decanted into a photo-electric colorimeter tube and read in the instrument. By means of a factor previously derived from a standard

solution, the concentration of the quaternary ammonium salt in the sample is calculated.

Epton's method appears to the author to be more convenient than the Auerbach method which involves particular care in the preparation of the coloured solution. It is simpler and quite useful for the higher dilutions used in practice. This method has been found useful and successful for single chain trimethylammonium salts, pyridinium salts and twin chain dimethylammonium compounds, and is given below with details for the estimation of a solution of 1 part in 10,000 of di-*n*-decyldimethylammonium bromide in water.

Ten c.cs. of a 0·0005 M. sodium lauryl sulphate solution were introduced into a 100 c.cs. stoppered measuring cylinder. To this were added 25 c.cs. of solution containing 0·003% Methylene Blue (B.P. quality), 1·2% sulphuric acid, 5·0% sodium sulphate (anhydrous) followed by 15 c.cs. of chloroform. The measuring cylinder was shaken with just sufficient force to ensure that the phases mixed thoroughly. The solution of di-*n*-decyldimethylammonium bromide, whose concentration had to be determined, was added at about 2 c.cs. at a time with intermittent shaking. When the colour of the upper layer began to become blue the rate of addition was reduced. The end point of the titration was reached when both layers, viewed in reflected light, were of the same colour.

The formula for calculating the concentration of di-*n*-decyldimethylammonium bromide for all dilutions is:

$$C = \frac{A \times B \times D}{N}$$

where

C = concentration in grams per litre.
A = molarity of sodium lauryl sulphate solution.
B = Number of c.cs. of sodium lauryl sulphate solution.
D = molecular weight of di-*n*-decyldimethylammonium bromide.
N = Number of c.cs. of di-*n*-decyldimethylammonium bromide.

Gravimetric methods involving the formation of insoluble precipitates between the quaternary and another compound have not achieved much prominence, mainly because of the difficulty in coagulation or filtration, and sometimes because of the instability of the complex formed. Another important qualification must be the certainty of obtaining a stoichimetric precipitation in this kind of test.

A method involving the formation of chloroplatinates was developed in the author's laboratory but presented too many difficulties for successful and consistent results. It is, however, interesting to compare results obtained on six samples of di-*n*-decyldimethylammonium bromide using two argentimetric methods, firstly, with potassium chromate as an indicator and secondly, using an electrometric titration, thirdly, precipitation of the quaternary as a chloroplatinate and fourthly, the Epton variation of the Auerbach method.

The results obtained by the four methods are tabulated below under the following headings:

Method A. Using potassium chromate indicator and titrating the bromide with 0·1N silver nitrate.
Method B. Electrometric method of Callan and Horrabin.[14]
Method C. By precipitation of the quaternary as a chloroplatinate.
Method D. Titrimetric method using an anionic surface-active compound (Epton's method).

Sample		% Quaternary Ammonium Compound by Method			
		A	*B*	*C*	*D*
1	..	55·5	56·0	53·6	58·6
2	..	47·4	47·9	48·6	50·6
3	..	47·4	47·4	43·5	50·0
4	..	48·5	49·0	44·8	50·0
5	..	56·0	56·0	52·2	57·9
6	..	60·4	60·1	46·1	56·1

One of the better methods utilising an insoluble precipitate is the ferricyanide method mentioned by DuBois[15] in a survey of chemical testing methods. The procedure is as follows:

One g. of the high-molecular quaternary ammonium compound is dissolved to 100 c.cs. with water in a volumetric flask. 25 c.cs. of this solution are transferred to a 100 c.cs. volumetric flask, and 5 c.cs. of buffer (260 g. sodium acetate and 250 c.cs. 36% acetic acid made up to 1 litre with water) and exactly 50 c.cs. of 0·01N potassium ferricyanide are added. The solution is made up to 100 c.cs. with water and allowed to stand for one hour with occasional shaking. It is next filtered through a dry No. 50 Whatman paper and the first 20 c.cs. of filtrate are discarded. Exactly 50 c.cs. of the subsequent filtrate are transferred to a 250-c.cs. flask, and 5 c.cs. potassium iodide and 5 c.cs. dilute hydrochloric acid are added. After one minute, 10 c.cs. of 10% zinc sulphate are added and the solution is titrated with 0·01N sodium thiosulphate, using starch as the indicator near the end-point.

The field test methods are a simple, if not necessarily accurate, means of checking the strengths of quaternary ammonium solutions when in use. Dye impregnated papers which change colour on immersion in solutions of these compounds have been developed, the degree of colour change giving an approximate index of the concentration. Tetrabromfluorescein has been used for this purpose, but in common with many test paper methods, cannot be said to be very reliable. Opinions as to the degree of colour change in the paper can vary quite considerably. Some test papers recently produced in the United States are supposed to be able to distinguish between solutions of 100, 200 and 300 parts per million; possibly further work will produce a range of test papers wherein a decided colour change will enable the concentration to be determined with more certainty.

Auerbach[16] has suggested a possible method utilising in principle the drop pipette method of determining surface tension. Since a solution of a cationic germicide will reduce the surface tension of water, the higher surface tensions will be found in the higher dilutions of the germicide. Therefore, if it is necessary to see whether a solution of one part of the germicide in 5,000 parts of water has lost some of its active material through use, a 1 c.c. pipette can be used, and the number of drops given by 1 c.c. of the solution from the pipette counted. The number of drops given by a freshly made up solution of 1 part in 5,000 in water would also be known, and if there is not a sharp decrease in the number of drops, then the concentration may be assumed to be still satisfactory.

Gain and Lawrence[17] have developed a turbidimetric method which involves the addition of 1 drop of undiluted normal horse serum to 1 c.c. of the quaternary solution to be tested. The contents of the tube are then agitated, and after half a minute it is examined for turbidity. If the turbidity is marked, then the solution may be between 1 part in 1,000 and 1 part in 2,000 of water. If of moderate turbidity, 1 part in 4,000; if there is only a trace of turbidity, 1 part in 6,000 and no turbidity will be found in dilutions higher than this.

A method recently developed in our own laboratories is based on the fact that the foam produced by a foaming quaternary ammonium germicide will be more persistent with the higher concentrations. This foam is destroyed by using spots of a 5% solution of Nesslers Reagent, the number of drops required to destroy the foam giving the concentration of quaternary. The method and results when used with di-*n*-decyldimethylammonium bromide are as follows:

Ten c.cs. of the di-*n*-decyldimethylammonium bromide solution to be tested are placed in a tube so that the 10 c.cs. level is nearly half-way up the tube. The tube used for the experiment was 2 centimetres internal diameter and about 9 centimetres long. The rubber bung is lightly fitted and the tube is shaken briskly. A stable foam will then form, and the 5% Nesslers Reagent, which is kept in a blackened or dark bottle fitted with a dropping stopper, is then added drop by drop until the foam, which is produced by shaking the tube, breaks down completely within 30 seconds. This may mean that a ring of small bubbles still remains round the edge of the tube, but these are ignored and the reading is taken from the breaking of the general mass of the foam. The number of drops required to break the foam is related to the concentration of di-*n*-decyldimethylammonium bromide in the following table.

TABLE VII

Number of drops . .	5	8	11	15	19	23	26
Concentration of di-*n*-decyldimethylammonium bromide	1:9000	1:6000	1:4500	1:3500	1:3000	1:2500	1:2000

A method which holds considerable promise as a field test, is that suggested by Harper, Elliker and Moseley,[18] and this consists of the formation of a red precipitate on the addition of 1 c.c. of quaternary solution to 1 c.c. of an eosin yellowish indicator and subsequent titration to a colourless compound with a standardised anionic surface-active agent. This method is said accurately to determine as little as between 10–20 parts per million of the quaternary ammonium compound in the lower range, and up to 500 parts per million in the higher range. The method lends itself to a very convenient field test merely by carrying out the titration with a dropping bottle instead of with a burette, and because of its value it must be included here. The reaction apparently takes place only over a relatively small range of *p*H and therefore it is necessary to use a citric acid buffer. The reagents are made as follows:

1. Indicator solution:
   - (*a*) Dissolve eosin yellowish dye (dye concentration of about 90%) in acetone (analytical grade) at the rate of 0·1 mg. of dye to 1 c.c. of acetone.
   - (*b*) Add acetone-eosin solution to tetrachlorethane at the rate of 1 c.c. of acetone-eosin to 9 c.cs. of tetrachlorethane.
   - (*c*) Remove the reddish colour from the solution by adding citric acid crystals (analytical grade) at the rate of 5 mg. of crystals to each c.c. of dye solution.
   - (*d*) Shake for one minute.
   - (*e*) Filter through Whatman No. 1 or equivalent grade of filter paper.
2. Buffer:
   - (*a*) Prepare a 5 per cent. solution of citric acid (analytical grade) and adjust to *p*H of 3·5 with N/10 NaOH. About 1·5 c.cs. of NaOH are required per c.c. of citric acid. Add 1% of tetrachlorethane to prevent mould growth.
3. Anionic solution:
   - (*a*) Make up a 0·01% solution of active anionic compound from 10% Fisher Laboratory Aerosol (10% di-octyl sodium sulfosuccinate) (1:1,000 dilution of the 10% Aerosol). Duponol paste has also been found satisfactory for this purpose.

Procedure

1. Place 1 c.c. of the solution to be tested in a test tube.
2. Add 0·1 c.c. of buffer solution.
3. Add 1 c.c. of dye solution. Two distinct layers will form.
4. Shake until a pink colour appears in the lower layer. The upper layer will be clear and should show no colour. About three to eight seconds of shaking are required.
5. Titrate with anionic solution. Add slowly and shake until colour fades out and lower layer becomes white. Quantity of quater-

> nary ammonium compound is indicated by the quantity of anionic compound required to remove red colour from the lower layer. Standards may be prepared from solutions of known concentration and a standard reference curve may be established.

Eosin yellowish is, of course, the dye known chemically as tetrabromfluorescein, and in addition to the method described above, these authors suggest making it the basis of a test paper method. This is done by making an acetone solution containing 0·025% eosin yellowish and 0·0025% of dichlorofluorescein. Two grammes of citric acid crystals are added per 100 c.cs. of solution. This is shaken for 1 minute and filtered through filter paper. Whatman No. 1 filter paper is saturated with the dye solution and allowed to air dry. The paper test gives a reddish colour in the presence of quaternaries; the intensity is roughly proportional to the concentration of the solution.

Quaternary Detergent Mixtures

Earlier in this chapter reference has been made to the mixing of quaternary ammonium compounds with detergent substances. Certain preparations are finding their market both in the United States and in this country. In formulating such preparations, the following considerations are important:

Firstly, to decide the compatibility of the detergent substances with the particular quaternary ammonium compound to be used; secondly to decide what proportions of all the constituents will produce the desired effect, and lastly, to recommend the correct concentration to be used in aqueous solution for a particular task.

The object of the quaternary detergents is to provide efficient cleaning with a considerable measure of destruction of micro-organisms, the principle being that mildly alkaline detergents normally cannot have much destructive effect upon bacteria, because the temperature at which they are used is never sufficiently high, and the caustic alkalinity is also too low to effect any destruction of micro-organisms.

The first function of a detergent is to remove unwanted matter from the surface to be cleaned, and this must be taken into the detergent solution and kept there in a suspended condition. If the detergent is really efficient, greases and oils will be emulsified and solid particles will be deflocculated and kept in this state of suspension. That quaternary ammonium compounds do assist in emulsification processes has been demonstrated in many places, and consequently a detergent containing a proportion should have desirable powers in this direction. Further, owing to the surface-active nature of the material, it will also have good wetting power. The soil which is being removed will, of course, in any food plant, be contaminated with micro-organisms, and the presence of the

quaternary ammonium compound in the detergent solution, will effect the destruction of a considerable number of these, thereby preventing the solution from becoming excessively contaminated with micro-organisms as would otherwise be the case. It may well be argued that, if the detergent is efficient, the question of whether the germs taken into the solution are killed or not, is relatively unimportant, but this statement, although on the surface having some degree of justification, does not bear close inspection, because practical conditions are known where it can be demonstrated that it is important to prevent the growth of germs in the detergent solution. An instance of this can be briefly described.

The ordinary automatic bottle-washing machine for washing returned glass containers in many industries, invariably has a detergent and then a warm rinse tank over or through which the containers next pass after the detergent wash. Infected residues are, of course, washed into the detergent solution. If the germs are not destroyed, they are carried over with drainings into the warm rinse section which is at the best temperature for encouraging bacterial growth, so that there is a rapid development of infection in the warm rinse tank which makes itself felt throughout the rest of the machine, and often causes infection in the final mains rinse pipes.

Numerous cases of badly washed bottles from the bacteriological point of view are directly traceable from this warm rinse infection; therefore, it is important that the organisms washed into the detergent solution from the containers should be killed as rapidly as possible.

With detergents based on caustic soda, it is important that the temperature should be maintained at least at 140°F., and that the strength should be not less than 1% in order to ensure destruction. The following table by Hobbs and Wilson[35] shows the time, temperature and concentration relationship for the destruction of 25% spores of *B. subtilis* by caustic soda.

TABLE VIII

(Hobbs and Wilson)

Temperature °F.	*Minutes*				
	1	2	4	8	16
120	2·44%	1·64%	1·10%	0·74%	0·50%
130	2·15%	1·44%	0·97%	0·65%	0·44%
140	1·90%	1·28%	0·86%	0·58%	0·39%
150	1·66%	1·12%	0·75%	0·51%	0·34%
160	1·46%	0·98%	0·66%	0·45%	0·30%

Although admittedly the destruction of spores is far more difficult than the destruction of vegetative organisms, the table

shows quite clearly the important relationship between the three factors. This is one of the main reasons why high caustic alkalinity is considered important in bottle-washing, although recently milder detergents containing a quaternary ammonium compound with special non-foaming properties have been brought into use. Here the dependence upon temperature is by no means so great, and makes an important safeguard, while carry-over into the warm rinse tank ensures repression of growth by virtue of the bacteriostatic effects referred to in the previous chapter.

This use of a quaternary detergent is discussed in more detail in Chapter VI.

Where it is important from the point of view of hand cleaning, that a detergent shall be of a mild nature, the value of using a quaternary ammonium compound is much enhanced, and ordinary hand dish-washing has proved itself to be an excellent field for this type of product.

The detergent substances with which quaternaries generally can be mixed are sodium carbonate, sodium sesquicarbonate, trisodium phosphate in some cases, and non-ionic wetting agents of the type produced by the reaction of ethylene oxide with fatty alcohols. The extent to which silicates and sodium hexametaphosphate can be used, and these are two of the most important substances in detergent formulation, depends upon the particular quaternary ammonium compound. It is all a question of compatibility, and this has to be regarded from two points of view.

As we have already noted in this chapter, visual incompatibility does not necessarily mean complete inactivation of the quaternary; but where the question of cleaning is concerned visual incompatibility means the formation of an oil or a precipitate which will have no detergency effect and may retard the whole detergency process. Certainly it will prevent a quaternary ammonium compound from exerting emulsifying and wetting powers, and may create a complex which may tend to adhere to the surface and complicate the whole cleaning operation. At the same time, as the previously tabulated results have shown, these complexes may still exert an anti-bacterial effect, provided that they are in suspension, but under practical conditions it is much more likely that inactivation will occur because the condition in the solution will be complicated by the presence of other soils, grease and dirt. Therefore it is most important to examine thoroughly the properties of any preparation of quaternary ammonium compounds containing substances other than those mentioned above, particularly silicates and polyphosphates.

Certain quaternaries may form complexes which are re-dissolved or broken up by sodium carbonate solution, so that in this case formulations containing the incompatible constituents may be rendered safe by the presence of an excess of sodium carbonate. Soaps and anionic wetting agents can generally be dismissed entirely from the list, as very few conditions will be found in which they

can be rendered compatible, although an interesting note has appeared in "Nature" by Matalon, Salton and Cohen,[36] showing that sodium lauryl sulphate can be prevented from forming a complex with cetyltrimethylammonium bromide by the addition of an equimolecular proportion of octyl alcohol, it being postulated that sodium lauryl sulphate preferentially forms a complex with octyl alcohol, and this prevents a complex formation between the anionic compound and the quaternary ammonium salt.

The question of hardness depositions in alkaline detergents which do not contain polyphosphates and are being used in hard water, is of course a problem of some difficulty unless a quaternary compatible with the polyphosphate can be found. In the presence of sodium carbonate, di-*n*-octyldimethylammonium bromide is such a substance, and its compatibility with silicates under the same conditions is also of the greatest value.

Formulations at present on the market are in powder form, except for those which are mixtures of quaternary ammonium compounds with non-ionic wetting agents.

An interesting feature of the quaternary detergents is that there is less evidence of strong adsorption of the quaternary to surfaces, both in the presence of alkaline substances and non-ionic wetting agents, better rinsing of the surface being evident.

Finally, a word must be said about the use of the quaternary compounds themselves as detergents. When used in a sufficient concentration which, of course, varies with the type of compound from 1% to 3% or 4%, the detergency properties are quite appreciable, but are not equal to anionic wetting agents at the same strength. On the other hand, the fact that quaternary compounds are more expensive than anionic wetting agents really eliminates them from active competition with the anionic wetting agents on the grounds of economy. Here again it is necessary to qualify the general statement by saying that in the United States a quaternary preparation is sold as an actual cleaner, but it is unlikely that any similar preparation will find a place in the British market unless the economics of quaternary ammonium compound production change quite considerably.

CHAPTER V

THE BACTERIOLOGICAL TESTING OF QUATERNARY AMMONIUM COMPOUNDS

THIS small book is not intended to be a review of a very considerable literature which has accumulated during the past few years, but it is realized that bacteriologists working in the fields of food and beverage production and sanitation, may be interested in a review of testing techniques as applied to these compounds.

The officially accepted standard for the evaluation of disinfectant substances in this country is the Rideal-Walker test, and in the United States of America the F.D.A. technique is very similar. In the Rideal-Walker method the organism *E. typhi* is used as the test organism and a standard solution of phenol is used as a basis of comparison with the substance to be tested.

Basis of Rideal–Walker Test

A series of dilutions of the disinfectant to be tested is made and corresponding dilutions of the phenol solution. It is important that these dilutions be prepared with care and accuracy. Then 5 drops of a 24-hour broth culture of *E. typhi* are added to 5 c.cs. of one of the dilutions of the disinfectant. Subcultures, with a standard platinum loop, are then made into broth at $2\frac{1}{2}$-minute intervals, up to 15 minutes. All the dilutions of the disinfectant and of the standard phenol solution are tested in the same way and at the same time. The subcultures are incubated at 37°C. for three days and the presence or absence of growth noted and tabulated. From the table so made, the dilution of the disinfectant which does the same work as a certain dilution of the standard phenol solution can be seen, and the result of the test is expressed by dividing the dilution of the disinfectant by the dilution of phenol, the figure so obtained being known as the "phenol coefficient".

Various modifications of this method have been suggested and are in use in various places, but nevertheless the above description is the basis of all these modifications of the test. The purpose of the Rideal-Walker test, it will thus be seen, is to compare any disinfectant substance in solution with a solution of phenol, one of the earliest known disinfectants. It does not take into account any particular or peculiar properties that the disinfectant substance may have, and does not distinguish between any degrees of killing power or of bacteriostatic effect. When preparations of quaternary ammonium compounds began to appear on the American market as disinfectants, their F.D.A. phenol coefficients, which are the equivalent of the Rideal-Walker coefficients, as published on the label, were seen to be very sensational, the stronger concentrations of these

bactericides claiming phenol coefficients often as high as 200 or 300 in comparison with 5 or 6 for the ordinary phenolic disinfectant. It was quite obvious that the reaction of the established American disinfectant market to the new compounds would be one of alarm, in view of the great difference in apparent activity, and it is probably this, as much as an academic interest, which prompted investigations to be made into the mode of action and killing power of the quaternary ammonium compounds. In 1946, E. G. Klarmann and E. S. Wright[19] published a paper in which they stated that the F.D.A. phenol coefficient method was entirely unsatisfactory for this type of compound, and resulted in a high figure which was entirely false and which rendered an economic disservice to other and older established disinfectants.

Their work suggested that the fictitious coefficients largely resulted from the following causes. Firstly, the carry-over of quaternary in the subculture resulted in bacteriostatic action so that tubes appeared with no growth, when in point of fact the organisms had not really been destroyed, and secondly, that the surface-active cations caused clumping of bacterial cells on the walls of the medication tube, and that these cells were, in fact, still alive. Therefore, the removal of the sample for subculture could not carry out with it the proper proportion of viable organisms.

They suggested two techniques which would overcome the disadvantages of the F.D.A. phenol coefficient method and present the true killing power of the quaternary ammonium compounds. One of these was a filter paper technique, and the other a semi-micro variation of the original F.D.A. method. These two techniques reduced the F.D.A. coefficients to rather less than one tenth of that originally claimed for the quaternary ammonium compound examined. Due perhaps in some degree to the method of presentation of the experimental data, the effect of this paper was to create the impression that the primary activity of quaternary ammonium compounds was bacteriostatic, and that the actual killing power was relatively small.

There is no doubt that this publication served to give a temporary check to the development of the new class of disinfectant. In spite of this, however, it also performed a very useful function in drawing attention to the limitations of phenol coefficient testing, and was instrumental in promoting an intensive search for new methods which could be applied to the quaternary ammonium compounds, without causing objections of the type raised by Klarmann and Wright.

Another factor also enters into the testing of the quaternary ammonium compounds, and that is in high dilutions, adsorption of the surface-active cations on glassware will reduce the effective concentration of the solution, as will also any other kind of surface, so that the filter paper technique of Klarmann and Wright was in turn criticized by A. S. DuBois[20] because of adsorption reducing the

effective concentration of the quaternary. The comments of Bernstein, Epstein and Wolk[21] on this question of testing are considered worth reproduction here. They say:

The mode of action of quaternary ammonium compounds on bacteria is not the same as phenol. . . . The quaternaries are a wonderful new tool. Many are odourless, non-irritating, non-toxic and perfectly applicable to the most exacting food plant. Most are detergents, a few being extremely effective as cleansing media.

Phenolics cannot make this claim. Why then compare the action of quaternaries against phenol? Each has its own sphere of influence.

The chlorine industry, by nature of the material itself, has never been worried about either the phenol coefficient of chlorine or the laboratory methods for evaluation. As a result that industry has established what 100 p.p.m. or 200 p.p.m. or 50 p.p.m. will do in certain operations, and even the U.S. Public Health Service recommends and accepts this type of standard for dishwashing, dairy sanitation, etc. The buyers and sellers of hypochlorite materials have consequently been trained to buy and sell on a concentration basis.

In other words, it is of more importance for users of germicides to know what the material they are buying will do in their plants, and in what dilution, than to have the phenol coefficient data of the material. After the proper concentration has been established for a particular sanitising job, all the user or the inspector has to worry about is a quick method for checking the strength of the solutions for purchasing and use specifications.

Investigations went forward in the following two or three years and many interesting points came to light. On the question of the clumping of living organisms by the quaternary ammonium solution on the glass walls of the medication tubes, DuBois[20] remarks as follows:

When the cationic germicides are added to bacterial suspensions, or *vice versa*, there occurs an adsorption of the cations on the bacteria, which neutralises their electrical charge. In the absence of a charge, the bacteria tend to adhere to one another to form a clump composed of a large number of bacteria, which may remain in suspension for a time before it migrates to the wall or bottom of the tube. This migration is probably not an instantaneous occurrence. However, the agglutination itself may, and probably does, take place very rapidly. The clump thus formed is surrounded by a solution containing the residual cations. However, only the bacteria forming the outside layers are in direct contact with the cations. Hence there arises a condition where the outer bacteria in the clump are killed, while those trapped in the interior are not, simply due to the fact that the cations have not reached them in sufficient amount.

If, however, a longer period of contact is allowed, the cations will gradually penetrate the clump and consequently reach the inner bacteria. When a sufficient concentration has been absorbed on the inner bacteria, the cations will be able to exert their full effect on these.

Another important point arising out of this is the actual percentage of organisms involved in the clumping phenomenon. In many of the methods published as modifications of the F.D.A. phenol coefficient technique, no indication is given of the sizes of the inoculum in numbers of organisms. Consequently the clumping phenomenon might be a serious objection or a trifling one.

It will thus be seen that the difficulties in applying the phenol coefficients to quaternary ammonium compounds arise because of (1) bacteriostasis, (2) adsorption of the quaternary and (3) clumping of cells in the exposure mixture, and work has been generally directed to finding methods which would eliminate most, if not all, of these objections. Attention was soon turned to the possibility of adding to the growth medium, some inactivating agent which would prevent any carried-over quaternary from exerting a bacteriostatic effect, and while it would not be possible to review the whole of the literature dealing with the various modifications, there are a few which may be briefly described. Mueller, Seeley and Larkin[22] in describing their method, introduce a useful refinement in that a definite size of inoculum is used and an inactivator is added to the growth medium, while a motor-driven agitator is designed to prevent clumping. A summary of their method is given below:

List of the various steps in procedure:

1. Wash vegetative cells or spores from agar slants into phosphate buffer solution. If spores are desired, heat to destroy vegetative cells.
2. Homogenise suspension.
3. Determine turbidity of suspension with spectrophotometer.
4. Standardise suspension to an established number of organisms per c.c.
5. Place 99 c.cs. of the desired strength of the germicide in a two- or three-neck flask and place in constant temperature bath. Add organic material or other substance if desired.
6. Start motor-driven agitator.
7. Add 1 c.c. of inoculum to germicidal solution.
8. Withdraw 1 c.c. of solution after definite contact time and transfer to a solution containing the inactivator.
9. Make necessary dilutions, plate, incubate and count according to Standard Methods.
10. Report results as percentage kill or percentage survival.

In the field of inactivation, soaps and anionic wetting agents at once suggested themselves as possible reagents for this function. On the other hand, phospholipids recommended themselves because of their known antagonism towards quaternary ammonium compounds. The characteristics of a good inactivator are, that in small amounts it must be positive and rapid in action, and this, without being itself germicidal or bacteriostatic. The main objection to the use of soaps and anionic wetting agents is that in the quantities they may have to be employed, they themselves can produce some degree of bacteriostasis. Further work has also brought out another valuable point: an inactivating agent which appears satisfactory for one quaternary ammonium compound may be unsatisfactory for another of different chemical constitution. This

means that unless published work definitely shows that a certain activator is safe for a particular quaternary, then thorough tests must be made in order to ensure that the activator really is efficient with that particular germicide.

In the author's laboratory, there has been great difficulty in finding a satisfactory inactivator for the twin chain compounds, di-*n*-octyldimethylammonium bromide being particularly unsatisfactory in this respect. So much so, that in order to eliminate bacteriostasis, it was found necessary to use a heavy inoculum of perhaps 50,000,000 or 100,000,000 organisms per c.c. and after exposure, use a dilution technique which would result in the amount of quaternary which was finally carried into the petri dish medium, being so low as to be outside the possible limits of bacteriostasis, thus, between 1 part in 5,000,000 and 1 part in 10,000,000 of quaternary in the solution could not possibly exert bacteriostatic effect. The method used for the testing of di-*n*-octyl and di-*n*-decyldimethylammonium bromides, is given below:

Five c.c. quantities of the quaternary dilution to be tested were placed in a series of sterile plugged test tubes. These tubes were placed in a rack in a cold water bath and allowed to reach the temperature of the bath (about a quarter of an hour). To each tube 1 c.c. of a suspension of the organism was added, the suspension corresponding to an opacity tube. The remaining bacterial suspension was then used to determine the accurate count of organisms used in the experiment, and this was done by diluting 1 c.c. of the suspension to a 1 to 100,000 level, and then plating 1 c.c. of this. Very good agreement was given by this method with the opacity figures for the experimental suspension. The tubes were rotated in the racks by hand to ensure thorough mixing during the time of exposure, which was 10 minutes in all cases. At the end of this time, 1 c.c. was withdrawn from each tube and diluted firstly to a 1 in 10,000 level, and with a second c.c. to a 1 in 100,000 level, and 1 c.c. of each of these dilutions was plated. Thus, if in the tube the quaternary compound was present as a 1 in 1,000 dilution, after exposure it would be diluted to 1 in 10,000,000 or 1 in 100,000,000 which dilutions are well outside the limits for bacteriostasis in culture media. The dilutions were plated in milk agar and the plates incubated for 48 hours at 37°C.

This method is not free from the objection that in the final dilutions before plating, cells which are not destroyed may possibly adhere to the glass of the vessels in which the dilutions are made, although every attempt is made to overcome this by agitation. On the other hand, it is an advantage that the actual exposure mixture contains very great numbers of organisms, so that the test solution has a harder task against the very much greater mass of organic material.

Another method which has been developed in this country by G. E. Davies[23] involves the use of a non-ionic wetting agent as a

dispersing compound to prevent the clumping of the micro-organisms. This method has been developed for the testing of cetyltrimethylammonium bromide.

Technique

(*a*) Preparation of Inoculum

Pipette 0·5 c.c. of a 24-hour broth culture of the test organism on to 5 c.cs. of nutrient agar contained in a 50 c.cs. conical Pyrex flask. After 24 hours' incubation at 37°C., wash off the growth with 10 c.cs. of sterile distilled water, using a few glass beads to loosen the growth and break up large clumps. 1 c.c. of this suspension is used as t inoculum. It will be found that viable counts remain fairly con ant from day to day.

(*b*) Test

Add 1 c.c. of the inoculum to 9 c.cs. of a dilution of the quaternary compound and mix well. After the desired period of contact, stir the mixture with a pipette and transfer 1 c.c. to a 1-oz. McCartney bottle containing 5 c.cs. of a sterile 1% solution of Lubrol W. and about 200 glass beads 2 mm. in diameter. Shake the bottle by hand for 1 minute and allow to stand for a further 4 minutes. (Bactericidal action ceases immediately the quaternary compound comes into contact with the Lubrol W.) The 5-minute period is allowed so that adsorbed compound may be removed from the bacterial cell. The shaking with glass beads and Lubrol W. serves to break up the clumps of bacteria. Plate four ten-fold dilutions by placing 1 c.c. of each dilution in a sterile petri-dish and adding 15 c.cs. molten agar. Count the number of colonies developing after 24 hours' incubation at 37°C.

Determine the initial inoculum by diluting the culture first in Lubrol W. glass beads and then in water to give plates representing 10^{-6}, 10^{-7} and 10^{-8} of the initial population.

Note: A separate pipette must be used for each manipulation, or confusing and inaccurate results will be obtained.

This author found by this method that cetyltrimethylammonium bromide had little effect on spores as far as actual killing was concerned. He also shows that clumps are broken up by the particular method recommended. The quaternary ammonium compound used was found to be a powerful bactericide even in the presence of moderate amounts of organic matter.

It cannot be too strongly emphasised that generalisations are dangerous in many ways when talking about quaternary ammonium compounds, and where new methods are devised to overcome any of the testing difficulties outlined above, it is particularly important that the chemical name of the compound with which the tests have

been carried out should be given as it does not follow, by any means, that the method can be generally applied.

Use dilution techniques have also been developed in the attempts to find more satisfactory methods of evaluating quaternary ammonium germicides. The idea behind this is to test the germicides at the dilute solution or dilutions at which it is intended for use rather than to use some arbitrarily chosen standards of strength which might be dictated by the laboratory method such as the phenol coefficient method. A typical instance of a use dilution technique following that broadly given by Mallman and Hane[24] consists of immersing small cylindrical pieces of glass into an inoculum of some particular test organism. These are then removed carefully and are dried lightly. They are next immersed in the solution of germicide of a strength which will be comparable to that actually in use, and then the cylinders are removed one by one at various intervals of time and immersed in a culture medium to which has been added a material calculated to inactivate any residual quaternary carried over. The time of exposure necessary to produce no growth in the growth medium will give an indication of the length of time of exposure necessary for destroying a certain organism at a certain concentration.

The objection to many methods, including the one briefly outlined above, is that they are qualitative and not quantitative, thus a solution which might destroy a culture of *S. aureus* at 50,000 organisms per c.c. in five minutes, might not successfully destroy all the organisms in an inoculum of 5 millions per c.c. in ten minutes, so that it is considered by some workers to be better to use methods which give an idea of the percentage survival or percentage kill.

Another objection to all kinds of laboratory testing may be made: whereas data concerning activity against one test organism can be obtained in the laboratory, it may have little relation to practical results actually obtained in use, because in practice a very mixed flora will be found and not merely one variety of one species. Arguing along this line can only lead to one conclusion, and that is that results obtained in practice are the only ones which are capable of demonstrating the real value of the particular disinfectant in any particular set of circumstances.

In a paper dealing with attempts to overcome many of the testing objections, Pressman and Rhodes[25] were of the opinion that negative results obtained from tubes into which medication mixture has been inoculated, does not necessarily mean that a 100% kill was obtained.

The interest in inactivators has become quite extensive in the last three or four years, and accordingly a table is included overleaf showing some of the main substances used for this purpose. Where possible the names of the quaternary ammonium compounds against which the substance was tried are given.

TABLE IX

Quaternary	*Inactivator*	*Author(s)*	*Reference*
Alkyldimethyl-benzylammonium chloride	Sodium decyl sulphate Sodium taurocholate	Baker, Harrison & Miller	*J. Exptl. Med.*, *74*, 621
Five compounds used Names not stated	Sodium oleate	L. H. James	*Soap and Sanitary Chemicals*, XXIII, No. 11 (1947)
Cetyltrimethyl-ammonium bromide	Sodium stearate	Pressman & Rhodes	*Soap and Sanitary Chemicals*, XXII, No. 4 (1946)
Various quaternaries. Names not given	Lecithin and Tween 80	Quisno, Gibby & Foter	*Am. J. Pharm.*, *118*, 320
Alkyldimethyl-benzylammonium chloride	Suramin sodium (Bayer 205) Sodium lauryl sulphate Lecithin and Tween 80 Sulphonated alkylaryl polyether alcohol	Weber & Black	*Soc. Am. Bact.* (Abstract of Proceedings 47th General Meeting p. 44)
Names not stated	Bacto oxgall	Klarmann & Wright	*Am. J. Pharm.*, *120*, 146 (1948)

Quite a number have been claimed to be satisfactory under the conditions used, but it is certainly recommended that the papers referred to be given the closest study before adopting any of them as a regular means of determining the efficiency of any quaternary ammonium compound. A method by L. H. James[26] not only claimed to be able to overcome bacteriostasis in the medication tube, but was also calculated to eliminate losses on the glass surface. The method used is briefly as follows:

1. Dilutions of the disinfectant are placed in medication tubes, generally in 5·0 c.c. amounts.
2. The test culture is added in 0·5 c.c. amounts, or proportional to the amount of disinfectant.
3. The disinfectant-culture mixture is held in a constant temperature water bath as usual.

4. An amount of sterile 0·2 % sodium oleate equal to 10 times the amount of disinfectant present (on a weight basis) is added to the medication tube mixture.
5. The walls and bottom of the medication tube are swabbed with a sterile cotton swab.
6. 1 c.c. and dilutions thereof are inoculated into petri plates and poured with agar.
7. After incubation at 37°C. for approximately 48 hours, the numbers of colonies appearing on the plates are counted and the number per c.c. of the mixture determined.
8. The total number surviving in the entire medication tube mixture is computed.

The procedure (designated for convenience the "oleate-agar method") was used in determining the numbers of bacteria surviving 10-minute exposures to five quaternaries, a cresylic disinfectant and phenol.

It has been observed that no kind of inhibitor or inactivator is added to phenol preparations when they are tested, and if the same procedure was adopted with mercurials, they would be found to be useless as disinfectants.

Bacteriostasis is in practice a valuable thing, and a powerful secondary factor making for the all-round efficiency of these compounds. The testing of quaternary ammonium compounds, under practical conditions, or rather the assessment of results obtained after their use, can, as a rule, be carried out by two methods, the use of the swab or a sterile rinse. In the use of these germicides in the food industries, surfaces of various kinds are primarily concerned, and where it is required to know the bacterial population of any particular surface, whether the substance be metal, glass, plastic or any other material, the use of the swab is the best means of assessing it. It is true that swabbing techniques can be criticized on the grounds that no two persons swab in the same way, and that the thoroughness with which the swab is squeezed into the Ringer solution varies with the individual, and that further, the condition of the surface of the plant or equipment being examined may condition, firstly, the percentage of organisms actually removed on to the swab, and, secondly, the numbers of organisms which will remain adhering to it and which will not be properly transferred to the Ringer solution. Thus, if the surface still holds a film of grease, then the adherence of the grease containing the residual bacteria to the swab will be an adverse factor.

In spite of these objections, swabbing techniques are the most frequently used, and on the whole it must be said the most satisfactory.

Rinsing techniques are far more uncertain and often much more difficult in manipulation, and only a few particular types of vessel or equipment lend themselves most conveniently to a rinsing technique.

It should be mentioned that before swabbing is carried out, it is advisable to rinse the surface of the equipment thoroughly with fresh sterile water. This will solve a great deal of the problem of overcoming bacteriostasis by removing most of the traces of quaternary ammonium compound from the surface to be swabbed. If this is done, it may not be necessary to add any particular inactivator to the growth medium and to this extent, at any rate, practical work simplifies the problems which have been discussed in this chapter.

PART II

APPLICATIONS IN THE FOOD AND BEVERAGE INDUSTRIES

CHAPTER VI

THE DAIRY AND ICE CREAM INDUSTRIES

THE importance of clean milk is so great that no measures which can be taken at any stage in its production and distribution to ensure a safe product must be neglected, and further, the importance of keeping quality is almost a parallel concern.

The cleansing and sterilising of all plant and equipment connected with this great industry have long been a matter for interest and research work by chemists and bacteriologists, whose efforts have kept pace with the ever-increasing measure of control upon which the authorities have insisted. For many years sterilisation after the cleansing process was confined to the use of steam and boiling water, and it was not until 1943 that permission was given for steam to be supplemented in England and Wales by the use of chlorine in the form of solutions of sodium hypochlorite. A number of brands of sodium hypochlorite received official approval, and these are quite extensively used in the English dairy industry.

At the time when this book is being prepared (1951), quaternary ammonium compounds have not received official approval for use in the dairy field in this country. Before such approval can be given, it is evident that the authorities must be entirely satisfied as to their efficiency and as to the manner of their use, and this may involve work which can easily take two years.

It is a great temptation to assume that results obtained in one field can be repeated in another. For example, the quaternary ammonium compounds have been used quite successfully in the catering industry in this country and America, and it is easy to assume that similar concentrations and times of exposure will be equally effective in dairy work. There is also a tendency to extrapolate results obtained in the laboratory into practical conditions by assuming, that if a concentration of 1 part in 4,000 of quaternary ammonium compound will kill a certain micro-organism in five minutes, then that compound is suitable for, say, the rinsing of milking machine equipment. Nothing could be further from the truth, and that is why it is most important to test the quaternaries intended for this purpose under the conditions which will prevail in actual practice.

In the dairy industry there are two main sections, firstly the producers, and secondly the processing or retail dairies. The producers mainly have problems with milking equipment, pails, coolers and other farm utensils. Some of these may, as far as the condition of the surface is concerned, be very poor. The processing dairy which takes the milk from the farm and grades and pasteurises it, is a very different proposition, the larger organisations being particularly well controlled and operating usually a most efficient system. Plant is generally well cared for and defective parts quickly replaced. It is hardly possible to get the same sort of thing on a farm, and consequently one must anticipate a wider variety of conditions on the producer side than in the processing and retailing section of the industry.

The authorities must also consider the possibility of the quaternary ammonium germicides being added to milk by unscrupulous persons in order to produce a fictitiously high keeping quality; that is to say, by using the germicide as a preservative. For many years this was one of the main objections to the use of hypochlorite solutions, and it was overcome in the end by the addition of a small quantity of sodium chlorate to the hypochlorite solution, thus enabling the sterilising agent to be easily detected in milk, although the hypochlorite itself was rapidly taken up by the milk, and after a short while could not be detected by smell or taste.

With the quaternary ammonium compounds, a similar sort of problem might arise, if these substances could be added to milk in such a manner as to act as a preservative, reducing the bacterial flora and thus prolonging the keeping quality of the milk. If, at the same time, these compounds were adsorbed on to globules of milk fat, detection of the fraud might be very difficult indeed. This question, however, does not seem to be a serious one since the work of DuBois and Dibblee[29] seems to indicate that in order to have any effect on the bacterial flora in milk, such large quantities would have to be added as to render the process uneconomical. These workers found that, in the compounds under review, it was necessary to use a concentration of 1 part in 500 of the quaternary in the milk, in order to produce even a temporary check in the growth of the bacterial population. Even this effect was relatively short-lived, and it seems reasonable to assume that the adsorption inactivation of the quaternary by the milk fats and proteins took place quite rapidly. Nevertheless, authorities concerned in this matter may not be satisfied that the substances could not be used for the purpose, and may insist upon the addition of some detectable substance which will enable the addition to be readily detected.

The position in Great Britain generally may therefore be said to be obscure with regard to the future of these compounds in the dairy industry, but in the United States of America and Canada considerable work has been carried out in an endeavour to assess the value of the new germicides for this purpose. Two of the most

important questions which must be asked when a new compound is being introduced for any purpose are (*a*) what advantages does it offer over existing sterilising substances, and (*b*) will the total cost be less?

Chlorine is well established in the dairy industry, and although its corrosion powers are considerable and storage and stability matters for care, it would appear to be reasonably well suited to its purpose. It has already been mentioned that chlorine can be readily absorbed by milk, and therefore the question of any taint or taste being left behind does not appear to arise. It is also a fact that solutions of chlorine can be used cold, and therefore, apart from the problem of corrosion and the question of repeated use, the case for the quaternary ammonium compounds seems to come down to one of cost.

Where the solution can be stored and used several times for disinfection treatment, the economic factor is reduced very considerably, and can even be made to show an advantage over chlorine solutions, which can be used only once.

A considerable number of micro-organisms met with in dairy work are spore formers, and the question of the destruction of spores must necessarily arise. In common with other disinfectants, the quaternary ammonium compounds only destroy spores with difficulty. On the other hand, high bacteriostatic activity against thermoduric spore formers isolated from milk cans, has been observed. Elliker[30] noted that hypochlorites were more efficient under farm conditions against *Esch. coli* than the quaternary ammonium compounds he studied, and noted also the resistance of *Ps. fluorescens* and *Ps. aeruginosa* to the quaternary compounds used. Good results have been obtained when using quaternaries for the disinfection of teat-cups at a strength of 400 p.p.m., the destruction of the *Streptococcus agalactiae* being over 99%. The use of these solutions on the teats of cows has also been successful, and further has commended itself by lack of irritation.

For the farm utensils, American literature also reports satisfactory results with the use of quaternary ammonium compounds. Conditions on farms are not always the most satisfactory or suited to the use of anything but the most simple type of treatment, thus, after use, a milk pail may be rinsed firstly in plain water, and secondly given a scrub or brush with detergent solution and finally a quick immersion in some kind of sterilising solution, usually hypochlorite. This immersion is only for a matter of a minute or so, and therefore the action of the disinfectant must be rapid. To those who know something of conditions on the average English farm, whatever may or may not be the practice of their American counterparts, doubts at once enter the mind as to whether the time of exposure to a reasonably strong solution of economical strength will be sufficient to produce the desired results where quaternary ammonium compounds are used, while any attempt to modify the

routine to suit the new sterilising agents is likely to be foredoomed to failure. On the other hand, to increase the strength of the solution so that it will compete in its rapidity of action with the usual hypochlorite solution, may well rule these compounds out of court economically, and there is no question whatsoever of storage and re-use of the solution on the average farm. Perhaps better prospects can be entertained for the use of quaternary detergent mixtures for cleaning and sterilising, since some results published by Elliker, Keesling, Miller and Wilster[31] suggest that they have possibilities, but even here the pre-use final rinse of the utensils was carried out with a hypochlorite solution.

The impression to be obtained from work so far published is that on the producer side of the dairy industry the most likely use for the quaternary ammonium compounds will be in the washing of teats and the disinfection of the teat-cups of milking machines; always bearing in mind that rubber may need to be watched for any developing stickiness.

Mallman[32] carried out an extensive investigation on the disinfection of milking machines and used 50 producers for this purpose, comparing detergent hypochlorite procedures with detergent quaternary ammonium solutions. He claimed that the quaternary ammonium germicide method produced counts that were less than half of those obtained when using the other procedures.

When the processing or retail dairy is considered, the possibilities for development are much greater, and one can see no reason why quaternary ammonium solutions should not be used with the greatest efficiency.

The milk is brought by lorry in cans, usually of ten-gallons capacity, and on arrival at the dairy it is checked, and in certain cases is returned to the producer as unusable. The milk is transferred from the can into a weighing tank and the can is then washed. There are a variety of can-washing machines in use, some of them using the sequence pre-rinse, detergent spray, hot-water rinse and steam. In some cases the contact time with steam is insufficient, and here the assistance of a sterilising compound may be very helpful. In America certain organic acids have been used in solution, being injected into the steam, and very satisfactory bacterial counts have resulted. Quaternary ammonium compounds might perhaps be used for this purpose in the hot water rinse, provided that this is not too easily wasted by carry-out of hot-water. One of the disadvantages of can washing is very often that loss of all the solutions occurs rapidly, but this is not always the case and most machines are capable of slight modifications.

The tank into which the milk is originally transferred must, of course, receive daily cleansing and disinfection, and bearing in mind the necessity for economy, it appears that after cleaning with a suitable detergent and rinsing off with plain water, a final spraying of a quaternary ammonium solution at a strength which might vary

between 200 parts per million and 500 parts per million according to the substance would be useful. In the author's experience, spraying of a quaternary solution on to the walls of large vessels is very effective and at the same time the most economical manner of utilisation. The sprayed solution could then be allowed time of contact limited only by the necessity for the re-use of the tank for milk. It is actually doubtful whether there would be any need for a final rinsing while the long period of contact would not affect the surface adversely in any way.

Pipelines from the tank to the pasteurising equipment are, of course, dismantled each day and thoroughly cleaned and brushed with a detergent solution and then sterilised either with steam or with a chemical sterilising agent. In this case, it might be possible to treat a very large section of the plant as one unit, and fill the pipes and plant with a quaternary solution and allow it to remain overnight, afterwards draining off and recovering the solution for further use. It must be emphasised that in all these procedures it is a *sine qua non* that thorough cleaning with a detergent must precede the sterilising function.

For the holder type of pasteurisers it will be necessary to resort to the spraying method suggested for the weighing tanks, but for the H.T.S.T. plant there is no reason why this, with its relatively small capacity, should not be included in the system and incorporated in the general overnight sterilisation.

Naturally, the H.T.S.T. plant itself must be thoroughly cleaned with adequate preparations to ensure that the plates are in a good condition of cleanliness and do not contain any film which might not only harbour bacteria, but inactivate the quaternary solution used.

To the fillers there is, of course, a further pipeline section, and the fillers themselves in the majority of cases can be conveniently sterilised by chemical solutions. The variation in design of fillers necessitates some caution in making general recommendations, but where possible an arrangement should be made to have bottles filled with the sterilising solution from the filler, so that the tubes are completely immersed for at least half an hour in that solution.

Here again, for the remainder of the time the overnight resting period could be used. It is one of the advantages in favour of quaternary ammonium solutions that where it is possible to give a long period of contact, they prove their efficiency and value.

Concern about corrosion under practical conditions can be dismissed, particularly where dairy plant, now consisting very largely of stainless steel, is concerned; other surfaces such as tinned steel and aluminium are also safe.

The long period of contact will ensure a really thorough disinfection enabling, at the same time, complete penetration of all crevices to take place. Rubbers on fillers must again be watched for any sign of stickiness which may develop, and if this point is watched regularly little trouble will be encountered.

From the other end of the dairy come the bottles into which the processed milk is filled. Mechanical bottle-washing is the general rule, and normally strongly caustic detergents are customary in these machines. Those usually encountered in the dairy either operate on a completely jetting principle, or else a combination of that with soaking, the former type being known as a "hydro" and the latter as a "soaker-hydro".

The use of surface-active materials in the hydro is very much restricted, if not altogether impossible, on account of the masses of foam which are produced, but detergents containing the quaternary ammonium compound di-*n*-octyldimethylammonium bromide have been used successfully for the cleaning of milk bottles, the principle being that the destructive power of the detergent against bacteria does not depend upon caustic alkalinity and temperature, but upon a positive sterilising agent. To a certain extent this reduces the dependence on temperature which is often a point of doubt in many bottle-washing processes, in which a fall in temperature of the detergent tank may result in inadequate destruction of the micro-organisms washed into the solution, with resulting contamination of the rest of the machine. The necessity of maintaining a high detergent temperature with a caustic detergent often results in a high percentage of bottle breakages due to thermal shock, particularly in winter, so that the use of a detergent which does not require such temperatures will help to overcome this trouble.

The presence of the quaternary has a powerful bacteriostatic effect on the subsequent warm rinse section of the machine, and this makes for a greater degree of safety. The effect of the use of this type of quaternary ammonium bottle-washing detergent can be demonstrated by the following table, which resulted

TABLE X

(Resuggan and Davis)

Swabbing points	*After Detergent "Y"*	*After "Q"*	*Percentage reduction in count after using "Q"*
Detergent tank wall	75,000	0	100%
Hot rinse tank wall . .	76,250,000	30,000	99·97%
Warm rinse tank wall	72,500,000	10,400,000	85·67%
Cold rinse tank wall . .	61,750,000	3,710,000	94·0%
Jets in hot rinse . .	Uncountable	0	100%
Jets in cold rinse . .	14,000,000	31,000	99·5%

from practical tests carried out in dairies by Resuggan and Davis[9]. Detergent "Y" was an ordinary alkaline detergent, and detergent "Q" was a detergent consisting of sodium carbonate, trisodium phosphate and 1% of di-*n*-octyldimethylammonium

bromide. The procedure was that the ordinary alkaline detergent was used for three days and swabs taken at the various positions named. Next, the quaternary ammonium detergent was used for a similar period, and areas nearby, obviously not exactly at the same points, were again swabbed. Other details of these experiments may be found in the same paper.

The addition of quaternary ammonium solutions to the warm rinse tanks of those bottle-washing machines which have them has also been mooted, and this is again a reasonable possibility for their use. It must be borne in mind that water is continually running from the warm rinse tank to the pre-rinse, and fresh water from the final mains rinse is continually entering, so that the dilution factor would appear to be quite important, and this of course will bring economics into the question. Where the quaternary detergent is used, however, the carry-over from the detergent section into the warm rinse is sufficient only to have a bacteriostatic effect, but even this, as results have shown, gives a decided improvement in the warm rinse section without involving the more expensive means of a drip feed addition of a quaternary solution to the tank.

Some interest has already been displayed in the possibility of quaternary ammonium compounds interfering with dye-reduction and other chemical tests which are applied to milk. Certainly it may be said that the substances have no oxidising or reducing power by themselves, so that it is not surprising to find that neither the resazurin or methylene-blue tests are affected by the small quantities of quaternary which could possibly enter milk. A very considerable quantity would be needed even to affect the intensity of colour, and so far in the author's laboratory it has not been possible to see any real difference where concentrations of 1 part in 1,000 of quaternary have been present in milk. Similarly, in the phosphatase test, negative results were obtained, as is shown in Table XI, so that from this point of view no objection can be raised against quaternary ammonium compounds.

Ice Cream Industry

The ice cream industry, although now under control in a manner similar to the dairy industry, has not had any limitations imposed upon it as to the type of cleaning and sterilising preparations which may be used. In this field also, hypochlorites are used in considerable quantity, and the sterilisation of ice cream plant and equipment is certainly a matter which calls for constant attention. Two main factors determine the nature of the chemical sterilising agent to be used, and here again they may be briefly stated as corrosion and cost. The cleaning and sterilising of ice cream plant is probably even more difficult than plant in a milk processing dairy, the residues being much more solid and more likely to adhere strongly to the surfaces concerned.

The need for thorough cleaning with a detergent is the same

TABLE XI

PHOSPHATASE TEST AND QUATERNARY AMMONIUM COMPOUNDS

Milk Quaternary Mixture	*L.B.U.*
19 c.cs. milk and 1 c.c. of 1 : 1000 di-*n*-octyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 2000 di-*n*-octyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 5000 di-*n*-octyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 1000 di-*n*-decyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 2000 di-*n*-decyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 5000 di-*n*-decyldimethylammonium bromide	< 2·3
,, ,, ,, ,, ,, 1 : 1000 bottle washing detergent containing dioctyl compound ..	< 2·3
,, ,, ,, ,, ,, 1 : 2000 bottle washing detergent containing dioctyl compound ..	< 2·3
,, ,, ,, ,, ,, 1 : 100 bottle washing detergent containing dioctyl compound ..	< 2·3

as in the dairy industry, and consequently the risk of inactivation of any chemical sterilant must be considered. Usually the most satisfactory procedure for ice cream plant and equipment is for a rinse with tepid water immediately after use, this loosening and carrying away most of the ice cream residues without the danger of hardening them on to the surface which might happen with hot water. The subsequent treatment with detergent solution which is carried out at a temperature of about 120°F with manual assistance where possible, is followed by a second water rinse which is essential, and then either steam or chemical sterilisation is carried out.

Using the quaternary ammonium compounds, the simplest method for large vats and vessels is to spray a solution of the quaternary at the rate of 1 part in 4,000 of water, according to the particular compound being used, and allow this sprayed solution to remain in contact with the surface until it is required to be used for the next batch. It is not even necessary to rinse before use in this treatment, and no adverse taint or taste can possibly be detected.

Freezers and ageing vats are not always amenable to steam sterilisation, and are most usually the subject for chemical sterilising. They often, however, present a more difficult cleaning problem and some degree of special care is necessary. Here again the spraying method for a quaternary solution may be considered ideal. Connecting pipelines, after being dismantled and thoroughly cleaned, can be immersed in a solution of quaternary of appropriate strength for half an hour or more if possible, and then reassembled

without any further rinsing. In some cases it might be found desirable to fill up certain portions of the plant and pipelines with the quaternary solution, the method in this case reducing in fact to a question of the cost involved.

Where possible the overnight soaking system will be found extremely efficacious, but the possibility of the re-use of the quaternary solution in the ice cream industry will depend very largely upon the cleaning efficiency.

Germicidal detergents containing mild alkalis and a quaternary ammonium compound, are coming into use in this field for the cleaning part of the routine, but it must not be assumed that the use of this type of product alone is sufficient to do both cleaning and sterilising. The fact is, that while the cleaning function will largely predominate, the detergent solution will, at the same time, be killing a large proportion of the micro-organisms, so that after its removal the condition of the surface will be bacteriologically better than if an ordinary detergent alone had been used. It will also mean that the subsequent sterilising treatment will be rendered easier. In some ice cream factories where ice cream bricks, choc ices and other confections are made, conveyor belts, usually of rubber, will be involved, and while spraying with a quaternary solution has been carried out successfully, it would appear to the author that scrubbing once a week with a detergent solution would be necessary to avoid a build-up of adsorption stickiness on the belt.

There is one particular aspect of the ice cream trade where quaternary ammonium compounds have proved themselves to be exactly the product for overcoming quite a difficult problem in sanitation. This refers to the service of bulk ice cream, which is usually carried out by means of scoops or servers, the ice cream being taken from a well which is surrounded by a freezing mixture or else is part of a freezing cabinet.

The scoops and utensils used for serving the bulk ice cream when not in use, are usually kept in a bowl or dish of water, and this frequently becomes a serious source of infection. A thorough examination of this problem and a practical solution to it was found by Davis, Resuggan and Ive[34] in results which have been published in the *Journal of the Royal Sanitary Institute.* It is nearly impossible to keep the scoops and servers free from contamination in the ordinary way, because they are continually being handled and then placed in the water until they are used next time. Here the water is the source of the trouble, rapidly becoming foul and growing bacteria which are often found to be definitely of a harmful nature, so that when the server is next used, the ice cream is inevitably contaminated. In the work reported, fourteen investigations were carried out in different ice cream bars or stalls, and the conditions were found to vary very considerably. In some cases, a sample of the rinsing water was taken after it had been in use for a period of time, and then the quaternary ammonium

solution was added and samples taken at intervals subsequently. An example of this is given by experiment No. 4 in the paper mentioned above, and this table is reproduced here.

TABLE XII

(Davis, Resuggan and Ive)

This investigation was carried out at a stall and on this occasion so-called "hot" water was in use, which was considered to be rather unusual, but apparently this was the common practice. Temperatures of the water when the samples were taken were therefore recorded. 3·5 per cent. quaternary solution was used at the rate of 20 c.cs. per gallon. A bucket containing 1 gallon of water was in use, and the number of ice creams served during time of testing was 130.

	Count per c.c.	*Coli (1 c.c.)*	*Temp. of water*
Water before addition of quaternary ..	2,060,000	Present	116°F.
Water ¼-hour after addition of quaternary	Nil	Absent	104°F.
Water ½-hour after addition of quaternary	200	Absent	98°F.
Water 1¼ hours after addition of quaternary	1,200	Absent	84°F.
Water 2¼ hours after addition of quaternary	7,000	Absent	70°F.

It will be seen that the count of the water was as high as 2,000,000 per c.c., and coliform organisms were also found to be present. After the addition of the quaternary, the count fell to zero with no coliforms, and even after a further 2¼ hours the count of the water was only 7,000 and the coliforms were still absent.

In another type of experiment the quaternary solution was added straight away to fresh water, and then service of ice cream carried on during a period of 3½ hours, at the end of which time there were nearly 3% of solids in the water so that the results obtained during the service of 150 ice creams were remarkably good. The table for this experiment is also reproduced. (Table XIII, p. 92.)

We have referred before to the problem of overcoming the bacteriostatic effect of quaternary ammonium compounds, and in this particular investigation the authors have attempted to measure the residual bacteriostatic effect under the actual conditions of the plating technique by addition of dilutions of pasteurised milk. This was chosen because it was felt that it would be likely to have a flora similar to that of pasteurised ice cream mix. Thus, by plating (1) pasteurised milk alone, (2) the server rinse water containing the quaternary ammonium compound alone, and (3) both the pasteurised milk dilution and the rinse water together, they were able to make an approximate measure of this residual bacteriostatic effect. The experiments showed that this residual effect resulted in about a 50% reduction in count. (Table XIV.)

TABLE XIII

(Davis, Resuggan and Ive)

This investigation was carried out in the kitchen of a canteen and here serving wells were in use. 3·5 per cent. quaternary solution was used at the rate of 15 c.cs. per gallon, i.e. 7·5 c.cs. to 2 quarts. The number of ice creams served during time of testing was 150.

Time	*Count per c.c.*	*Room temperatures*
12.05 p.m.—Fresh water	25	—
15 c.cs. quaternary solution added—		
12.15 p.m.	0	—
12.30 p.m.	0	—
12.45 p.m.	30	—
1.0 p.m.	100	—
1.15 p.m.	200	—
1.30 p.m.	200	80°F.
1.45 p.m.	200	—
2.0 p.m.	400	80°F.
2.15 p.m.	700	—
2.30 p.m.	700	76°F.
2.45 p.m.	1,000	—
3.0 p.m.	1,200	68°F.
3.15 p.m.	2,700	—
3.30 p.m.	3,000	—

Total solids in rinse water: 1.30 p.m.—1·35 per cent.
3.30 p.m.—2·93 per cent.

TABLE XIV

(Davis, Resuggan and Ive)

RESIDUAL BACTERIOSTATIC EFFECT ON Q.A.C. IN RINSE

	Colonies per c.c.		
	1	2	3
1 c.c. rinse containing Q.A.C.	0	30	20
1/100 c.c. milk	3,600	4,700	3,300
1/100 c.c. milk plus 1 c.c. rinse	1,000	2,900	2,300
Per cent. reduction in count	72	38	30
1/1000 c.c. milk	17,000	5,000	19,000
1/1000 c.c. milk plus 1 c.c. rinse ..	4,000	3,500	7,000
Per cent. reduction in count	77	26	73

CHAPTER VII

BREWING, SOFT DRINK AND ALLIED INDUSTRIES

In the beverage industries, quaternary ammonium compounds are finding one of their biggest outlets. They are being used in the production and bottling of beer and the manufacture of all kinds of soft drinks and fruit juices, and although not quite so important in this country, the production and bottling of wine.

The use of the quaternary ammonium compounds in the breweries has spread very considerably, replacing in many cases older methods of treatment. The main problem in the brewery is the keeping quality of the beer, and this would appear to be more important than the question of hygiene which is so predominant in the milk industry. Beer itself will not grow pathogenic organisms to any great extent, and the common disease germs which flourish so rapidly and well in milk and ice cream, soon die out when placed in an environment of beer. To this extent beer is regarded as a safe drink, and while there are particular problems of health involved in the serving of beer, no problem really arises in the course of its production.

In the past few decades, the extension of the bottled beer trade, as distinct from draught beer sales, has produced a demand by the public for sparkling bottled beers, and to produce these with the minimum of cost and trouble has been, and still is, one of the main concerns of brewers, since any beer which appears hazy when poured out, is apt to be rejected by the consumer as bad. In point of fact, there are still a few naturally conditioned bottled beers, the devotees of which know better than to apply the bright beer criterion, but these are in the minority, and by far the greater percentage of bottled beers in most countries must answer first of all the insistent demand for clarity. Pasteurisation is also extensively practised to attain this end, although the flavour of the beer is affected, and the process involves additional expense.

The best way of achieving clarity is to make sure, that when the beer is bottled, it contains no micro-organisms which are likely to multiply or which can carry on any further fermentation process, since this will inevitably lead to haziness and also possibly in certain cases, to some degree of off-flavour. Obviously, one of the first necessities is that the bottling plant shall be kept sterile, or at any rate as near sterile as possible, and the other is, that the beer shall be thoroughly filtered in such a manner that no micro-organisms can pass through the filter, which process is, of course, carried out before carbonation and bottling. In the average brewery, it is easy to demonstrate that a sample of beer, after passing the filter, contains very few micro-organisms. Further

samples taken through the plant at various points may not show, and usually do not show, such a good picture. The condition of the bottles from the bottle-washing system, must also be watched with the greatest of care, as this can be the cause of beers throwing hazes or deposits, even when the rest of the plant is in first-class condition bacteriologically.

The organisms which can be responsible for these troubles are mainly yeasts, either yeasts from the brewing room, or wild yeasts which infect the atmosphere in breweries; but certain kinds of ordinary bacteria also contribute towards these troubles, such as certain types of lactic acid bacteria and Acetobacter species, but the main causes of trouble are yeasts.

It has previously been mentioned that yeasts are more resistant to disinfection than are most kinds of ordinary bacteria, and consequently a great deal of care must be taken to exterminate them in places where they should not exist. The bacteria are also of types which do not succumb easily to chemical disinfection, and once again it is necessary to use higher concentrations than would be normally necessary in other industries.

Existing methods of sterilisation include steam, alkaline hypochlorite solutions, hydrogen peroxide and sodium bisulphite, and these methods have been used in breweries for many years. Great caution has to be observed in the use of some of these substances, as the susceptibility of beer to flavours of one kind and another is well known. The most thorough and careful rinsing has to be used after a hypochlorite or sodium bisulphite, and it is because of the comparatively low taste and odour factors of the quaternary ammonium compounds that they have been so well received in what is, after all, one of the most conservative of industries.

The importance of unaffected flavour of beer is probably equalled by the necessity to ensure the head retention of the beer, that is, the natural foam which appears when beer is filled into a glass.

While the average brewer, knowing very well that a filtered bright beer cannot contain much that is really of food value to the consumer, is sometimes cynical about the whole bright beer tradition, his reaction to the question of a good head on the beer is positive, and nothing will injure any product in the eyes of a brewer so much as its ability in practice to affect the head of foam. It must, therefore, be said at once that practically all surface-active compounds have some effect upon the head retention property of beer. Sometimes the effect is additive and increases the stability of the foam, but in the majority of cases, the surface-active substances would appear to act as foam depressants as far as beer foam is concerned. It can be demonstrated in the laboratory that any quaternary ammonium compound in solution, provided that sufficient is added, will depress a given head of foam from practically any beer.

In the chemical disinfection of brewing plant, it is almost a *sine qua non* that such treatment will be followed by rinsing with clean water, so that the possibility of interference with the head retention qualities of the beer may be dismissed. The matter has received far more attention in connection with the problem of the rinsing of drinking glasses in licensed premises, and although perhaps the proper place to discuss this matter in its entirety is in the chapter on the catering industries, the fundamental aspects of the action of quaternary ammonium compounds on beer, can be considered here.

Although the work of Bishop, Ward and Kloss[44] deals with drinking glass sanitation, their results on the action of quaternaries on beer foam are particularly interesting. Their calculations showed that effects on head retention could be produced by 1 part of the quaternary in 5,000,000 parts of beer, but they point out that even this quantity is relatively large when compared with certain other types of compound which destroy the head on beer.

These observations are sufficient to show the extreme sensitivity of beer head to the action of various depressants.

The method adopted by these authors was, in brief, to rinse the glasses in quaternary solutions of various concentrations, allow two minutes for draining and then to fill the glasses with beer by means of a standard and completely reproducible method: and it was noted that, with the quaternaries which were most surface active and had the best anti-bacterial powers, the life of the foam was least when concentrations between 1 part in 12,000 and 1 part in 400 were being used. At 1 part in 200, however, with the more surface active substances, the life of the foam began to lengthen and in fact became very pronounced, showing that at this concentration sufficient quaternary was left in the glass actually to assist the stability of the foam.

When considering surface activity, the rate of migration to interfaces is the predominant factor, and in the case of these results, it leads to an interesting thought. The glass is brought in contact with the solution of the quaternary ammonium compounds for presumably a few seconds only, and then allowed to drain for a few minutes. Therefore, the more surface-active and bactericidal quaternaries will adsorb more readily to the glass surface in that period, whereas in the case of the less active materials, less adsorption will take place and more will be removed in the draining process. It seems likely, therefore, that where the glasses were rinsed in the more active solutions, a greater degree of adsorption on the glass took place, so that at certain concentrations, activity against the head of the beer was more noticeable. If this is so, the differences recorded may be quantitative rather than qualitative.

In the glass rinsing problem under present conditions, as will be discussed in the chapter on catering, there is no question of a subsequent rinse, and a further point comes to mind in this connec-

tion, that the solutions of the more surface active materials with the higher powers and rates of adsorption, will probably not drain as quickly or as smoothly as those in which these characteristics are less predominant. Observations in the author's laboratory have also shown that different kinds of beer exhibit different susceptibilities to this action, some being more sensitive to certain quaternary ammonium compounds than others, while this order can sometimes be reversed with respect to yet other beers.

Obviously the constituents of the beer may, in some cases, tend to neutralise the quaternary as far as its surface active properties are concerned, in which case the more surface active quaternaries will be inactivated as far as foam depression is concerned more easily and to a greater degree than the less surface active compounds, so that the inversion of results which may have been obtained with other beers can be understood.

In view of this experience, it would seem that the only safe thing to say about the subject is, that one given quaternary ammonium compound has a certain effect upon the head of a certain beer at a certain strength and under certain conditions. Attempts to state that one quaternary ammonium compound is better than another without testing it against a large number of beers, appear to the author to be unwise.

Under practical conditions, it is very often difficult to notice any difference between a dry glass, a glass rinsed in plain water and a glass rinsed in a quaternary ammonium solution, and details of some practical tests which were carried out in the author's laboratory may be of interest. After the final pouring, it was assumed that no longer than four minutes would elapse before, under ordinary practical conditions, the glass would be raised to the lips of the consumer; in fact the actual time observed in licensed houses is often very much less. The tests were, therefore, carried out in the following way:

The glasses, dry, wet and wetted with quaternary solution, were prepared and then the beer carefully poured into ordinary dry glasses, endeavouring to be certain that the same amount of foam was present in each glass and the same amount of beer. When these were assessed to be the same, the contents were placed rim to rim on the prepared glasses, and using the same motion, poured into these glasses and condition and duration of the foam was then noted. This method of pouring beer was found to be much more satisfactory than pouring straight out of the bottles where, in the first instance, it is not possible to control the amount of beer and the amount of foam coming out from any one bottle. This test was intended to simulate practical conditions, and although variations, due to the human element, must exist, repeatable results were obtained.

Attempts have been made on the Continent to use quaternary ammonium compounds as beer preservatives for the very light and

lager type beers, which are common there. This follows the use of ethoxyethylbromacetic ester as a preservative in beer, but quaternary ammonium compounds have not been found satisfactory for this purpose, in that the amount necessary to produce preservative action also produces a slight haze in these beers. Although in the English beers this does not seem to occur to the same extent, there is no question of its use in this country as a preservative, since laws with regard to preservatives are quite strict, admitting only benzoic acid and sulphurdioxide. In beers the latter only is allowed.

Properly used for the disinfection of plant and equipment, there should be no question of the substances being left behind in a quantity likely to cause loss of head retention or of flavours.

Reference has been made in other chapters to the high powers of adsorption of these substances, and it is as well that this property should be borne in mind in certain disinfection tasks. A periodic cleaning with detergent solution is always desirable to avoid any considerable build-up of quaternary film, which may carry with it on the walls of the vessel traces of beer proteins and other substances. A case illustrating this came to the author's notice a little while ago: beer storage tanks which had been treated with quaternary solutions, by filling the tanks with solution as a regular treatment, developed a sticky film after a period of several months. On examination, this was found to consist of beer residues which formed an almost invisible layer on the walls of the tank by reason of an adsorbed quaternary ammonium film. It had not been the practice to clean out the tank at all with any kind of detergent, and hence the building up went on for nine months until it was possible to detect the sticky surface. The remedy lay in an occasional wash-out of the tank with detergent solution which would thus have prevented any such building up of deposit.

In most cases in the brewery cleaning is carried out prior to sterilising, so that this sort of problem does not arise, but in places where it is not customary to clean out, then the condition of the surface must be watched.

The main uses for quaternary ammonium compounds in the brewery are for all kinds of large vessels, vats and storage tanks, and unquestionably the most satisfactory method of applying them is by means of a spray. The solution requires to contain between 1 part in 2,000 and 1 part in 4,000, according to the quaternary ammonium compound being used and the recommendations of the manufacturers. Pipelines and beer mains provide another sterilising problem, and it is customary to clean these by circulating or pumping a detergent solution through and then disinfecting with a sterilising solution of quaternary ammonium compound. Here again the tendency is to allow a lengthy period of contact, very often overnight, and quite frequently over the week-end. Fillers are very often sterilised overnight and the method follows that described in the previous chapter.

The immersion of rubber hoses into solutions for sterilising requires caution with regard to the condition of the interior of the hoses. Where very old perished hoses are being used, the crevices and cracks will be full of beer residues often of very long standing, and the quaternary solution's first tendency, because of its surface-active and detergent properties, will be to loosen these and bring them away, exposing very often surfaces with unpleasant taints. Copious rinsing must, therefore, be carried out. Rubber hoses should also be watched to see that no stickiness occurs on the outside of the hose, although this is not a serious point and can be overcome by brushing this lightly with detergent solution.

One of the most important uses for these compounds is in the washing of screw stoppers which are still in use in a large number of breweries in this country. The usual method is for the stopper to receive a wash, either in water or in a weak detergent solution, for the purpose of removing debris, pieces of labels, etc. After a running rinse, the stoppers are deposited in a tub or tank containing quaternary ammonium solution, usually of a strength of 1 part in 5,000 to 1 part in 10,000, again according to the compound and the recommendations of its manufacturer. The stoppers remain in this vat until they are ready for use, when it is customary to remove them from the solution, shake them free of as much moisture as possible, and then to use them directly in the bottles without any further treatment. This method has proved to be most satisfactory, the solution tending to penetrate further behind the rubber ring on the stopper than a non-surface-active disinfectant, and having a really remarkable effect, as bacteriological results obtained by the author have shown. There would appear to be no danger of contamination of the beer by quaternary solution, as practical results have demonstrated over very long periods.

There are several kinds of filters used in brewery bottling stores, and since they all have the function of removing micro-organisms, care of the filters and their cleaning and sterilising is of the utmost importance. While the author understands that quaternary ammonium solutions have been used successfully in the sterilising of certain kinds of beer filters, it would appear that the vast area of a filter can adsorb a very large quantity of quaternary ammonium compound, so that there may be some danger of its almost complete removal from the sterilising solution, while the adsorption on to the filter may, in time, interfere with the functioning of this important part of the plant. This contra-indication actually arises from purely theoretical considerations, but they seem to be so well founded that beer filters are probably best left to other methods.

Solutions which have been used for sterilising in the plant can be used further for washing down walls in order to destroy mould growth. Several breweries, indeed, store and re-use their quaternary

solutions many times, depending upon the particular quaternary and its resistance to inactivation.

With regard to the bottles, very much the same applies to the bottle-washing machine in the brewery as in the dairy, and quaternary detergents are available now for brewery bottle-washing as well as for the washing of milk bottles.

The same observations with regard to the dosing of warm rinse tanks apply also equally well to brewery bottle-washing.

Tubs in which a quaternary solution of the usual strength for disinfection has been placed have been found very useful in the brewery for immersing utensils and appliances of a small size which are in constant use. These remain in the solution until they are ready to be used again, and after a quick rinsing are ready for their normal work. Almost complete sterility with these utensils can be maintained by this means, and the solutions, as a general rule, need to be changed not more than twice and very often only once a fortnight.

It would not be possible to leave the section dealing with the brewing industry without referring to the question of casks and the possibility of the use of quaternary ammonium compounds for deodorising casks which smell badly. The effective sterilisation of casks by quaternaries depends very much upon the interior condition of the cask and its age, and while good results have come to the author's notice, it is only fair to say that an almost equal number of bad ones have also been noted. Where inadequate steam sterilisation of the cask has been practised for some time, a very serious condition may arise, and very often the quaternary in solution will be absorbed into the pores of the wood and into other crevices, without being able to make an effective destruction of the micro-organisms, since the quaternary may be adsorbed on to the wood fibres and very largely inactivated. In those cases where deodorisation has been successful, the procedure has been to fill the cask with quaternary solution at a strength of 1 part in 1,000, and allow it to remain for a whole day, transferring the solution then to another cask and rinsing out the first cask with plain water.

The task of keeping wine vats in good condition during the months which often elapse between their use, has been considerably assisted by the use of these substances, the normal practice being to leave some wine in the vat. This is an expensive method, and the loss of wine by evaporation is often hastened by human assistance so that another method is desirable. Water used alone soon becomes foul with bacterial and yeast growth, but the addition of 1 part in 2,000 to 1 part in 4,000 of a quaternary germicide to the water, using this solution to fill the vat, enables it to be maintained in a satisfactory state during its resting period.

Problems in the soft drink industry are very closely allied to those in the brewing trade, and here again yeasts are probably the greatest difficulty. The syrups used for manufacture are rich in

sugar, and after dilution are readily fermented by yeasts which is a very undesirable characteristic in this industry.

Mixing pans must, of course, be thoroughly cleaned and then sprayed or sponged with a quaternary ammonium solution of 1 part in 2,000 to 1 part in 4,000 strength, according to the preparation being used and the manufacturer's recommendations. The film of quaternary thus produced should be left untouched until the mixing pan is required for use again, when it should be flushed out thoroughly with clean water.

Syrupers vary in design, but all should be subjected to a thorough cleaning before the sterilisation process. The syrup pans usually feed the syrup by gravity into the syruper through pipelines which may be of metal, glass or plastic, and it is possible to clean and sterilise the whole syruping system, firstly by means of a warm water rinse and then by a detergent solution which is put into the syruping pan and allowed to run through the whole syruper, this in turn being followed by a clean water rinse. The plant is then ready for sterilisation with the quaternary solution which should be of the strength indicated for the mixing pans. The time of contact should be as long as possible, and the syruper itself should be fitted with bottles to allow discharge of the sterilising solution through all parts. A final flushing with clean warm water should be undertaken immediately before use.

The filler can, of course, be dealt with in the same way as for breweries and dairies, but here the problem is not by any means so great. Nevertheless, yeasts can often find a home in the filler head, and carbonated water supplies have been found by the author sometimes to be quite heavily contaminated with yeasts. Therefore, it will be necessary to treat the fillers in the mineral water factories with just the same degree of attention as they receive elsewhere.

Screw stoppers are again very frequently met in the soft drinks industry, and these can be treated exactly as described for the brewery.

The checking of fermentation in stored fruit juices has been attempted using the quaternary ammonium compounds, and encouraging results were obtained, although this was purely an experiment and the regulations concerning preservatives would militate against this method being used in this country.

Needless to say, the environment in all food and beverage factories can be and must be kept clean and free from all kinds of contamination, and the use of germicidal detergents for cleaning, and quaternary ammonium solutions for sterilising, often as a final use before discarding the solution, is becoming quite a regular practice.

CHAPTER VIII

CATERING INDUSTRY

One of the most important uses for the quaternary ammonium compounds is in the catering industry, much of the earlier work with these compounds being carried out in this field in the United States, and a great many publications made in that country on this subject.

The great advantage of quaternary ammonium compounds in this field is, of course, their freedom from odour and relative tastelessness in use dilution. The use of chlorine, although very effective as a sterilising compound, does not find much favour because of the odour and the taint which even the smell of chlorine in the atmosphere can impart, if not to the actual foodstuff itself, at any rate to the palate.

The catering industry may be considered under the following headings:

1. The kitchen and preparation of food.
2. The returned crockery and cutlery and dish-washing.
3. The service of beer and drinks in licensed houses, and bars.

In the kitchen it is of the utmost importance that the most spotless cleanliness prevails, and unfortunately this is one of the black spots in the catering trade. Congested conditions, badly ventilated and inadequate space, are only some of the causes of unhygienic food preparation; labour difficulties and the necessity for continual economy are other factors which have served to increase the problem.

It can be seen from statistics how, during the war years, outbreaks of food poisoning have increased, and in spite of warnings from the authorities and newspaper campaigns, there seems to be little sign of any abatement.

The primary need is, of course, for education in elementary personal hygiene, and after this the most thorough cleaning and sterilising of everything which comes in contact with food for human consumption.

The rinsing of the hands in a quaternary ammonium solution is an excellent way in which to maintain the skin in a sterile condition for operators preparing food, and a routine of this type would go a long way to overcoming a difficult and objectionable problem. It has long been known that the quaternary ammonium compounds in solution, when applied to the skin, adsorb in a manner which provides a film, the surface of which is sterile and will destroy any micro-organisms with which it comes in contact. This effect has been made use of by surgeons and physicians in medical practice.

The next consideration, as far as health is concerned, takes the problem to the other side of the counter. The fact that a multitude of people in all conditions of ill-health, many of them with infectious diseases, use restaurants, brings the next and more difficult problem of preventing the dissemination of infection by means of crockery and cutlery. Everything will now depend upon the efficiency of washing and sterilising methods, if the infection which inevitably accumulates on used utensils is to be prevented from becoming a menace to public health. From recent observations of conditions in cafés, it would appear that two of the main sources of infection are forks and cracked cups. All crockery returned to the canteen is infected to some degree, but forks and cracked cups show the heaviest populations of disease-causing organisms.

The ideal method of cleaning the returned vessels would be first to remove all residues by an effective pre-rinsing with warm water, and then to pass them to a machine where a hot effective detergent solution, at a temperature of over 140°F., is sprayed in such a way that every part of the crockery or cutlery receives the full force of the spray for at least two minutes. From this section the objects will go forward to a final clean water rinse at 180°F., to which also they will be subjected to treatment for over two minutes, and afterwards a hot air dryer will finish off the job and leave the plates, dishes, cups and saucers and cutlery in a sterile and dry condition, without any need for contact with the human hand. This ideal for washing and sterilising is hardly ever achieved. In many instances the need to economise in space is against it, and fuel shortages have also played their part in recent years. In many canteens and kitchens, however, a decided approach has been made towards the ideal. The installation of efficient washing-machines has, without doubt, contributed to this advance. Even so, however, in nearly all instances the crockery is subjected to some kind of wiping with a cloth after the final rinsing. This is a most certain way to undo the good work which may have been done in the machine.

It is argued, with justification, that wet plates cannot be used for food service and hence the need for a final drying. Certainly something could be done to provide a clean sterile cloth which would be changed at reasonably frequent intervals. If the operator kept his or her hands in a good clean condition and paid strict attention to the rules of personal hygiene, the danger would be very much reduced. Nevertheless, the fact remains that the ideal would be to arrange for a hot-air drying. Where this has been attempted, however, the objection has been made that the utensils dry with a film of lime on them, often due to hard water, so that where hard water exists it should be softened before being used for the final rinse.

Existing methods of crockery washing in canteens and restaurants are divided into two classes. Firstly, there is the

hand-washing method which takes place at a wooden, metal or porcelain sink. The plates are roughly brushed free from loose debris and are immersed in a sinkful of hot water which contains a small amount of detergent, usually a mild one. The water is often around 100 or 110°F., and at this temperature cannot be expected to destroy any germs at all. As the temperature falls below 100°F., which is a little more comfortable for the hands of the operator, optimum temperature conditions for the growth of bacteria are achieved. Not only does the water rapidly become foul from waste foodstuff, but also the bacterial population rapidly increases. Plates, dishes and cutlery are also swabbed to free them of dirt, and the bacteriological condition of the swab is almost too bad to contemplate. Sometimes the plates are transferred to a second sink or to a compartment in the same sink, where running water is allowed to rinse them. This is, of course, an improvement, but only too often the rinse water is static, and for reasons of economy is only run off every now and again. Hence the rinse water soon becomes heavily contaminated. Utensils and implements are next dried with a cloth, again with no improvement to their hygienic condition.

Where there is only one sink for the washing-up and no facilities for subsequent sterilisation in boiling water, the quaternary ammonium compounds have an almost unequalled field for development. In the first instance, the detergent used in the washing-up water can be a balanced detergent, which contains a quaternary ammonium germicide in order to effect some degree of sterilisation during the actual washing-up process. This will show a considerable improvement over the ordinary soda type of detergent, and in an investigation carried out a short while ago, where ordinary washing soda was compared with a special detergent containing a quaternary ammonium compound, the results given in Tables XV and XVI were obtained.

These tests were carried out under absolutely practical conditions in an ordinary café kitchen, and the whole investigation was executed by a responsible bacteriologist in charge of an independent laboratory, that is, the manufacturers of the quaternary detergent had nothing to do with obtaining these results beyond providing the detergent. The results speak for themselves and show quite clearly the improvement which can be achieved by this type of material. The almost complete absence of coliform positives with the quaternary detergent was one of the most encouraging features. Admittedly, in a kitchen where only one sink is available, there is little opportunity for a hot drying oven, and therefore either the crockery is allowed to drain by itself, or else it is given a cursory wiping with a rather dirty cloth. Once again, the quaternary ammonium compounds can achieve an enormous improvement in this latter condition, which under ordinary circumstances can be very dangerous. An examination of a drying-up cloth which had

TABLE XV

ORDINARY DETERGENT

No.	Description of sample	Time sampled	Plate Count at 32°C. 3 days' incubation 1/10 c.c.	1/100 c.c.	1/1000 c.c.	Plate Count at 32°C. 5 days' incubation 1/10 c.c.	1/100 c.c.	1/1000 c.c.	Coliform at 37°C. 1 c.c.	1/10 c.c.	1/100 c.c.	1/1000 c.c.	Condition of Swab
1	Large plate	12.35	106	1		122	1		—	—	—	—	S.T.
2	Cup freshly washed	12.35	1	1		1	1		—	—	—	—	S.T.
3	Knife	12.40	2	1		5	1		+	+	+	—	S.T.
4	Spoon	12.44	35	90		35	95		—	—	—	—	V.T.
5	Small plate	12.46	1,400	272		1,400	276		—	—	—	—	V.T.
6	Fork	12.50	63	6		67	10		+	+	—	—	V.T.
7	Soup plate	12.55	1	60		2	96		—	—	—	—	S.T.
8	Small plate	1.00	400	42		400	60		—	—	—	—	V.T.
9	Plastic plate	1.03	1	Spr.		1	—		+	—	—	—	V.T.
10	Cup	1.05	236	84		240	120		—	—	—	—	S.T.
11	Large plate	1.07	164	20		200	25		—	—	—	—	V.T.
12	Plastic small jam dish	1.09	36	4		40	4		+	+	—	—	S.T.
13	Fork	1.11	3	1		10	1		—	—	—	—	V.T.
14	Metal knife	1.13	1	1—1 mould		2	1—1 mould		—	—	—	—	S.T.
15	Large plate before wiping	1.18	38	3		60	4		+	—	—	—	S.T.
16	Large plate after wiping	1.19	8	1		8	1		+	—	—	—	S.T.
17	Cup	1.27	264	33		288	34		—	—	—	—	S.T.
18	Cup	2.35	230	41		304	56		—	—	—	—	S.T.
	Detergent I	1.30	1,600	1,600		1,600	1,600		+	—	—	—	
	" II	1.30	1,600	480		1,600	490		+	+	+	—	
	Rinse I	1.30	1,600	130	1	1,600	134	2	+	+	—	—	V.T.
	" II	1.30	1,600	222	20	1,600	328	25	+	+	—	—	V.T.

S.T.—Slightly turbid.
N.T.—Not turbid.
V.T.—Very turbid.

Volume of rinse and swab rinse—20 c.cs.
Amount plated stated in column heading.

TABLE XVI

QUATERNARY AMMONIUM DETERGENT

No.	Description of sample	Time sampled	Plate Count at 32°C. 3 days' incubation			Plate Count at 32°C. 5 days' incubation			Coliform at 37°C:				Condition of Swab
			1/10 c.c.	1/100 c.c.	1/1000 c.c.	1/10 c.c.	1/100 c.c.	1/1000 c.c.	1 c.c.	1/10 c.c.	1/100 c.c.	1/1000 c.c.	
1	Dessert plate	12.23	2	1		2	1		—	—	—	—	N.T.
2	Cup	12.25	3	1		4	1		—	—	—	—	,,
3	Plastic saucer	12.27	5	1		48	12		—	—	—	—	,,
4	Large plate ..	12.30	6	1		8	3		—	—	—	—	,,
5	Plastic saucer	12.40	4	1		4	1		—	—	—	—	,,
6	Large plate ..	12.45	2	1		3	2		—	—	—	—	,,
7	Cup	12.47	6	5		7	6		—	—	—	—	,,
8	Fruit plate ..	12.49	1	1		1	1		—	—	—	—	,,
9	Cup	12.52	1	1		6	4		—	—	—	—	,,
10	Soup plate ..	12.56	1	1		2	1		—	—	—	—	,,
11	1 pint jug ..	12.59	1	1		4	3		—	—	—	—	,,
12	Cup	1.01	1	1		4	3		—	—	—	—	,,
13	Dessert plate	1.04	1	1		2	1		—	—	—	—	,,
14	Plastic plate	1.08	1	1		1	1		—	—	—	—	,,
15	Plastic saucer	1.10	1	1		1	100		—	—	—	—	,,
16	Large plate ..	1.13	1	1		1	1		—	—	—	—	,,
	Tank 1 ..	13.13	1,600	1,600		1,600	1,600		—	+	—	—	,,
	,, 2 ..	13.13	6 spr.	1		6 spr.	2		—	—	—	—	,,
	Rinse ..		2	1	1	2	1	52	—	—	—	—	,,

N.T.—Not turbid.
Volume of rinse and swab rinse—20 c.cs.
Amount plated stated in column heading.

been in use for some 20 minutes or half an hour, showed that it contained about a hundred million micro-organisms, many of which were harmful pathogenic types. When the cloth before use was treated in the following manner a great difference was noted.

The cloth was immersed in a solution of a quaternary ammonium compound, 1 part in 1,000 of water; it was allowed to remain for five minutes, and afterwards was wrung out and hung up to dry without any further rinsing. When this cloth had been in service for nearly half an hour covering the same washing-up period as the first cloth, a bacteriological examination revealed only about 20,000 organisms present, and none of them of the harmful type.

This is a really striking difference, and illustrates very well the point that there are certain tasks for which these compounds are admirably suited, and this may be said to be a very good example of that point. The cloth had been impregnated with the quaternary ammonium compound, and during the course of wiping up, the germ-laden moisture was transferred to the cloth and a local concentration of a quaternary ammonium solution was produced, thus destroying the germs.

This treatment has no injurious effect upon the cloth whatsoever, and in fact actually lengthens the life of the cloth by preventing the fibres from being attacked by acidic residues which may be picked up from one source or another.

The two sink system is, of course, a big improvement on the one sink, and here the second sink is used for a sterilising rinse. In order for this to be successful, the crockery must be immersed in really hot water, preferably nearly boiling, for two or three minutes. Unfortunately, conditions in the post-war world do not tend to guarantee an adequate supply of very hot water at the right temperature, and time and again temperatures fall below that necessary for proper sterilisation of the crockery. A margin of safety can be provided by keeping 1 part in 5,000 of a quaternary ammonium preparation in the rinse water. It can have no possible harmful effect, and should the temperature at any time fall, then sterilising will still be carried out by the presence of the germicide. In fact, the single rinse system could be supplemented by an ordinary galvanised bath or some other easily procurable tank, in which a quaternary ammonium solution is kept for this purpose.

The cleaning and scrubbing of all tables in the kitchen, and the whole kitchen environment, should be carried out by means of a sterilising detergent solution.

A considerable amount of dish-washing by machine is now carried out in the larger restaurants and hotels. These machines fall roughly into three classes. The first, essentially simple in construction, consists of two or more baths agitated by paddles, the crockery and cutlery being immersed in baskets which are removed from one section to another. The first section is a bath containing

rinsing water, the articles to be cleaned being placed in a wire basket or crate which is inserted into the bath. The solution is agitated and its temperature raised usually to 100 or 120°F. The basket and contents are then transferred to a hot detergent solution and the procedure is repeated. Next comes a very hot rinsing section, the water in this case being at about 180°F., and it is in this section where the quaternary ammonium solution should be used, although the use of a quaternary ammonium detergent in the detergent section will obviously be a valuable adjunct.

In the second class of machine, the dishes are sluiced with a detergent solution applied through a revolving arm, and this effectively treats the whole available surface. Next comes a hot water rinse at 180°F. applied by the same revolving arms in the same manner and in the same sluices. The difficulty with this type of machine is that continual dilution weakens the detergent solution, and this must be made up frequently. Here again a quaternary detergent could be used.

A third type of washing-machine is that which utilises the two tank principle. There is firstly, a detergent tank from which the solution is pumped through sprays above and below the articles to be washed. After they have been washed at the usual temperature of about 140°F., the dishes pass forward as each fresh tray is pushed into the rinsing section. Here, rinse water at a convenient temperature is pumped through sprays above and below the tray, so that all traces of detergent solution are rinsed from the crockery and cutlery before they pass through a final hot spray at a temperature of over 180°F. Here again the recommendation would be a suitable non-foaming quaternary detergent, and this could be relied upon by carry-over, to keep down the growth of any bacteria in the rinse section, if there is any tendency for this latter to gather a bacterial growth.

All cloths, swabs, etc., used in the kitchen for whatever purpose, certainly require boiling, and where possible a subsequent treatment in quaternary solution will, as has already been mentioned, go a long way towards maintaining them in a satisfactory condition.

The sterilisation or sanitisation of beverage glasses in licensed premises, has been also the subject of interest on both sides of the Atlantic. Here again attempts to use chlorine solutions have been rather unsuccessful because of the faint odour of chlorine which lingers behind the bar, and can often be detected by people standing on the other side. Further, in this country, the single rinse system is the common one in the public-houses, warm water being most frequently used, the temperature of this varying between 90 and 110°F. when first filled into the bowl, afterwards falling to a much lower temperature.

As a rule, glasses are continually in circulation while the premises are open for the sale of drinks, and after use are rinsed in the bowl and allowed to drain; they are then re-used as required. The period

of draining time depends upon the demand for glasses, and in some public-houses, when trade is slack, the glasses will be taken from the draining-board and dried with a drying cloth and then put aside on the shelves.

During rush periods at the week-ends, glasses are often quickly rinsed and filled up again immediately.

When the licensed premises are closed, the glasses are usually washed in warm water sometimes containing soda, and finally dried with a glass cloth before being placed on the shelves ready for the next period of opening.

Bacteriological examinations have revealed that the rinse water is a source of dissemination of bacteria. The bacterial counts are often quite considerable, and coliform organisms are usually found to be present. It is now generally admitted that the quaternary ammonium compounds are almost ideally suited for combating this problem, and at least two commercial preparations of quaternary ammonium compounds designed specially for this purpose, are now available to the licensee.

One of the most difficult problems is the frequency with which the rinse water is changed. This very often depends upon the pressure of work and the actual appearance of the rinse water. Nevertheless, numerous examinations carried out by Resuggan and Davis[37] showed that normally the water was changed well before 100 glasses had been rinsed, although even at this period the

TABLE XVII

(Resuggan and Davis)

Sample No.	*Temp. °F.*	*Plate count Blood agar*	*Coliform test*			*Description*
			1 *c.c.*	1/10 *c.c.*	1/100 *c.c.*	
NO BACTERICIDE USED						
	Cold					
1	45	32,000	+	+	—	New water
2	—	31,000	+	+	+	After 20 glasses
3	—	34,000	+	+	+	,, 40 glasses
4	—	39,000	+	+	—	,, 60 glasses
CONCENTRATION OF BACTERICIDE 1:30,000:						
5	Cold	193	—	—	—	New water
6	—	396	—	—	—	After 20 glasses
7	—	1,508	—	—	—	,, 40 glasses
8	—	1,200	—	—	—	,, 60 glasses
CONCENTRATION OF BACTERICIDE 1:15,000:						
9	Cold	139	—	—	—	New water
10	—	596	—	—	—	After 20 glasses
11	—	444	—	—	—	,, 40 glasses
12	—	562	+?	—	—	,, 60 glasses

quaternary ammonium compound was still effectively maintaining control of the bacterial population.

Table XVII shows the degree of contamination encountered, and the effect of the quaternary ammonium preparation upon it.

It will be seen from Table XVII, that with the quaternary content as low as 1 part in 30,000, a very important effect was obtained with the rinse water, but a preparation of 1 in 15,000 was found to be better. In point of fact, the actual concentrations now recommended are higher than these.

While this question of the effect on the rinse water is admitted to be important and indeed vital, the question arises as to whether the quaternary ammonium solution on the glass is disinfecting the actual rim of the glass where it has come in contact with the mouths of consumers. The following table shows very clearly the kind of result which can be anticipated, and that the length of draining time has an important influence upon the numbers of surviving organisms.

TABLE XVIII

(Resuggan and Davis)

Swab results for pairs of inverted glasses allowed to drain after rinsing at room temperature.

	Time of draining (min.)	*Interval between sampling and testing (min.)*	*Plate count (yeastrel agar) per c.c. rinse*	*Coli per c.c.*			
				1	0·1	0·01	0·001
Rinsed in water ..	2	5	5,910	+	—	+	—
		7	28,400	+	—	+	—
	4	8	6,300	+	+	+	—
		7	8,400	+	+	—	—
	6	8	158,000	+	+	+	+
		9	218,000	+	+	+	+
	8	9	227,600	+	+	+	+
		8	46,900	+	+	—	+
	10	5	96,200	+	—	+	—
		8	53,200	+	+	+	—
Rinsed in water containing quaternary ammonium preparation (1 : 7,500) plus 0·1% detergent ..	2	5	8,000	+	—	—	—
		7	18	—	—	—	—
	4	8	1,500	—	—	—	—
		10	245	—	—	—	—
	6	10	193	—	—	—	—
		11	195	—	—	—	—
	8	9	22	—	—	—	—
		10	9	—	—	—	—
	10	9	13	—	—	—	—
		11	2,150	+	—	—	—

Other work in this field has been carried out by Bunker[38] in this country, while similar results have been obtained in the United States of America by Mallman[39] and others.

Bunker's results are interesting and of relatively recent date, and show that the quaternary ammonium compounds are quite effective for this class of work. His examination of rinse waters again shows a picture of infection which can only be regarded as serious, more perhaps from the presence of coliform organisms than for ordinary bacterial count. The results obtained are very similar to those given by Resuggan and Davis in this respect.

TABLE XIX

(Bunker)

BACTERIAL COUNTS OF WATER IN SINK

No bactericide added

Sample taken	*Temp. of water °F.*	*No. of bacteria per c.c.*	*Coliform organisms*		
			1 *c.c.*	0·1 *c.c.*	0·01 *c.c.*
At start	116	7,220	—	—	—
After 10 glasses	103	14,360	—	—	—
,, 20 glasses	99	12,880	—	—	—
,, 30 glasses	91	13,600	+	—	—
,, 40 glasses	87	26,500	+	+	+
,, 50 glasses	84	27,000	+	+	—
,, 60 glasses	83	24,240	+	+	—

Bunker, in this publication, discusses the use of a special dispenser to be fitted to the sink for this purpose, and details of its construction are given, the idea being that every time fresh water is run into the bowl, the correct amount of quaternary solution is automatically added. Obviously such a useful device is desirable, in order to ensure that the disinfecting preparation is always present in the bowl.

CHAPTER IX

USES IN OTHER FOOD INDUSTRIES

Bakeries

IN bakeries, quaternary ammonium compounds are of some considerable value. Their properties of being odourless and relatively tasteless are here of particular importance. They can be used for combating mould growth in many places, and for the prevention of bad flavours which are due to mould and bacterial contamination.

Using always the manufacturers' recommended dilutions for particular tasks, the germicidal solution can be sprayed in the mixing-room, on walls, ceilings and equipment twice a week in summer and once a week in winter. In the dough-room, the troughs should be sprayed once a week, and the walls and ceilings of the room itself also disinfected. Proof boxes should also be sprayed twice a week in the summer and once a week in the winter. Proof boxes are often bad offenders due to mould and off-flavour development. In the wrapping-room the slicing machines and equipment should be sprayed, also table surfaces and utensils with which the finished product may come in contact; this should be done at least once a day. It is particularly important to spray rooms which contain stale bread or returned goods at least twice a week, as the chances of introducing moulds into the bakery in this way are great. The inside of all bread delivery vans should also be sprayed at least once a week. All other parts of the plant which may otherwise become sources of product contamination should be similarly sprayed.

Any locations where unpleasant odours are found should also be sprayed as frequently as necessary, since this is evidence of bacterial or mould growth which although it may not occur in any actually important production section of the bakery, would always pollute the atmosphere and help to spread infection generally.

In the bakery it is very important for all workers to keep their hands as near sterile as possible, and this can be done by wetting the hands and sprinkling a few drops of quaternary ammonium solution upon them and then rubbing this in.

All cloths, towels, proofer bags, etc., should receive regular impregnations with quaternary ammonium solution as has been described in the chapter on catering.

Cheese Factories

The question of sterilisation in cheese factories is by no means very acute; good cleaning should be the rule and general hygienic precautions should always be taken, but the fact remains that cheese factories do not present anything like the same problem as do

the milk processing dairies. One difficulty which is frequently met, however, is trouble with the starter organism, and this is usually due to the activities of the bacteriophages of the lactic acid streptococci. Prouty[40] has successfully used quaternary ammonium compounds to inactivate this bacteriophage. He examined the effect of six different quaternary ammonium compounds, and found that a 200 p.p.m. solution of these would inactivate the bacteriophage on a two-minutes exposure. Chlorine is also used for the inactivation of bacteriophages.

Generally speaking, any sterilisation considered to be necessary in the cheese factory is usually carried out by means of heat, as caution must be observed in the use of chemical disinfectants. Presumably, however, the spraying of walls, floors, benches and environment with the quaternary solution would be of value.

Treatment of Certain Fruits and Vegetables

The keeping quality of fruits often depends upon the extent to which the exterior is kept free from yeasts and moulds; and in food factories, where these are dealt with for canning, it has been found a useful procedure to include a quaternary ammonium compound in the pre-wash water. An example of this particular use is given by Bernstein and Epstein.[41] In this paper they discuss the pre-soak washing of cucumbers used in pickles. The spoilage of the cucumbers resulted from their contamination by aerobic spore-forming micro-organisms. These are present in large numbers when the cucumbers are received at the processing plant or salting station. Spoilage in salt stock is indicated by the development of off-flavour and a spotted and poor appearance. The contamination is so heavy, that although the pickles are subjected to pasteurising, this treatment is insufficient to eliminate the organisms, and consequently a very severe heat treatment is necessary in order to obtain sterility. Since it is not possible to control this treatment exactly so that all the batches received will be adequately sterilised, the only obvious approach is to reduce the initial count of the flora by some kind of semi-sterilising pre-treatment. Where a prolonged heat treatment had been used, the quality of the pickles was impaired by a loss of crispness. The quaternary ammonium compound was added to the solution in the pre-soaking tank, and although the exact chemical concentration is not stated, the commercial preparation was used at the rate of 1 oz. to 5 American gallons of water. From this pre-soaking tank, the cucumbers are transferred into a rotary washer where they are subjected to sprays of water for the removal of the germicide and dirt. After this washing, the cucumbers are ready for bottling and processing.

It is evident that this sort of treatment can be extended to quite a number of vegetables and fruit, and while it is not known if this is yet done in this country extensively, it is evidently a possibility for development.

Citrus fruits have also been similarly washed in solutions of quaternary ammonium compounds, and here there is no need to give any kind of rinse, and after drying off, the residual film of quaternary will have a protecting effect.

The treatment of vegetables which are to be frozen is also another processing in which the quaternary ammonium compounds are likely to be used; because whereas while in the cold state the keeping quality is safe, upon the food reaching room temperature, rapid attack by micro-organisms can take place, and the careful treatment of such foods, prior to being frozen, seems likely to be a possibility in overcoming this disadvantage.

Naturally, this aspect of the use of the germicide may very well depend upon the laws with regard to preservatives, or the authorities being perfectly satisfied about their use in this way.

Eggs

The washing of eggs before they are broken for processing has been another successful application of these substances.[33] Here again, as in most of the other cases, the work was done in the United States. The eggs which were often grossly contaminated with soil and dirt of one kind or another, carried a very high bacterial population which, as can be expected, included a large proportion of intestinal organisms. Therefore, the destruction of these by washing in a solution of a quaternary germicide, in order to avoid contamination of the egg once it is broken, was very important.

Food Shops

Wherever food is sold and can possibly come in contact with infection, it is important that every precaution should be taken to destroy such infection at every opportunity. The washing-down of counters, weighing and serving utensils, the impregnation of cloths and the spraying of trays and covers, are all subjects for an intelligent use of quaternary ammonium compounds.

Butchers' shops are particularly a subject, and in the United States the spraying of meat exposed for sale has been suggested and practised successfully. Fishmongers, too, can find a use for these preparations in the same way, and the sterilising of all implements as well as deodorising is to be envisaged as a use.

Refrigerators can also be kept free from infection by spraying or swabbing of the interiors, when upon occasions they are de-iced; the idea that a low temperature means absence of micro-organisms is, of course, often generally held by the laity, but this is not so, although the rate of growth depends upon the temperature of the cold store or refrigeration space. As an example of this, the bacteriological examination of cold-room space of an ocean-going liner was examined while the vessel was in the London Docks, one room being swabbed over one square foot of wall and in the other

battening was similarly swabbed and the rinse from the swabs plated. It will be observed that in the cold-room which had a temperature of 35°F., a considerable bacterial count was found, while moulds were very much in evidence. In the other case the temperature was 2°F., and here the bacterial count was very much less and 30 yeasts were obtained. This points to the value of the use of some satisfactory type of germicide, at any rate occasionally, for such cold storage and refrigerator space.

TABLE XX

Sample No.	*Temp. of room*	*Area swabbed*	*No. of organisms per c.c. on Milk Agar*		*Beerwort Agar (for yeasts and moulds)*	*Coli*	
			37°C.	22°C.	22°C.	37°C.	44°C.
I ..	35°F.	1 sq. ft. of wall	90,000	310,000	Heavy mould growth	Neg.	Neg.
II ..	2°F.	6 ft. of battening	320	290	30 yeasts	Neg.	Neg.

It has also been noted that ice in which fish is packed for transport will, if sprayed with a quaternary ammonium compound solution, materially protect the fish against bacterial action, and that it will keep in a better condition for longer periods. Similarly the washing of shell-fish has been carried out.

It will be seen from this final chapter that the instances of possibilities for the quaternary ammonium compounds could be multiplied very considerably, and any surface in the food and beverage industries which it is required to keep in a near sterile condition forms, with very few exceptions, a possible outlet for these substances.

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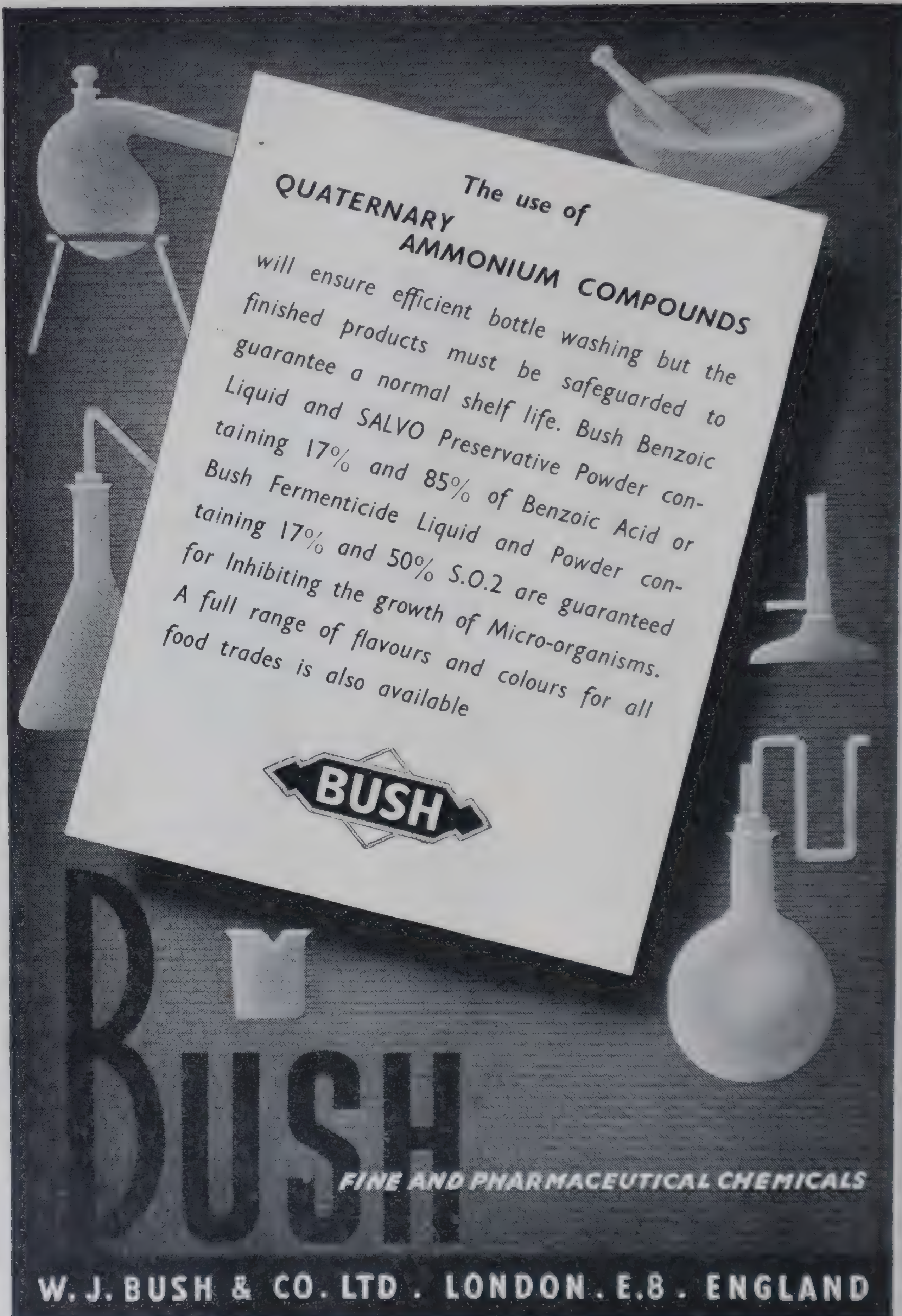
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